FOLIA MEDICA CRACOVIENSIA
Vol. LI, 1–4, 2011: 45–58
PL ISSN 0015-5616

KRZYSZTOF GIL, ANDRZEJ BUGAJSKI, BEATA SKOWRON, PIOTR THOR

INCREASED C-FOS EXPRESSION IN NODOSE GANGLION IN RATS WITH ELECTRICAL VAGUS NERVE STIMULATION

Abstract: *Increased c-fos expression in nodose ganglion in rats with electrical vagus nerve stimulation*

B a c k g r o u n d. Central nervous system receives information from the gut and modifies food intake mainly by vagus nerves. Some our data show that long — term electrical vagus nerve stimulation (VNS), which "mimics" satiety signal from gut, may cause reduction of body mass and decrease in food intake.

O b j e c t i v e. The purpose of this study was to assess the effects of chronic vagal stimulation on neurons in the nodose ganglions of vagus nerves, analyzed by c-Fos expression and image analysis.

M e t h o d s. Male Wistar rats (n = 24) were implanted with microstimulator (MS) and kept during the whole study (3 months) on high calorie diet. Sub-diaphragmatic left vagal nerve was stimulated by electrical rectangular pulses duration 10 ms, amplitude 200 mV, frequency 0.05 Hz generated by MS. Twelve rats (6 — control and 6 — MS implanted) were used for 3-week and 3-month experiments respectively. At the end of experiments the nodose ganglions of both vagus nerves (left and right) were taken, formalin fixed and paraffin-embedded specimens were made. The nodose ganglions neurons were identified by immunochemistry (PGP 9.5 as a marker) and the percentage of c-Fos positive neurons (anti c-Fos as a marker) were evaluated.

R e s u l t s. Assessment of c-Fos positive neurons in nodose ganglia of vagal nerve showed significant increase in percentage of positive cells in the left nodose ganglion (4.19%) and non significant in the right nodose ganglion (2.64 %) compared to control (1.44%) in 3-week experiment. Data obtained from 3-month experiment were similar: (4.97%; 2.66% and 1.68%) for left, right and control respectively. In both experiments number of c-Fos positive neurons was higher in left vagal ganglion compared to the right ganglion and control. There were no significant differences between 3-week and 3-month experimental groups.

C o n c l u s i o n s. Increase in c-Fos expression in left nodose ganglion neurons confirms the afferent transmission of the signal (generated by MS) from periphery to the brain by the vagal nerves.

Key words: c-Fos, nodose ganglion, vagus nerve stimulation, rat
Słowa kluczowe: c-Fos, zwój guzowaty nerwu błędnego, stymulacja nerwu błędnego, szczur

INTRODUCTION

Vagus nerves provide the major neuroanatomical link between gastrointestinal sites and the brain. Central nervous system (CNS) controls feeding behavior and metabolism after receiving information from the gut, modifying food intake. Gastric mechanoreceptors and jejunal chemoreceptors activated by food ingestion evoke electrical impulses transmitted by afferent fibers of vagus nerves into the CNS sites, to suppress feeding [1, 2, 3].

The left and right vagus nerves are formed by converging afferent and efferent rootlets as they leave the cranium through the jugular foramen. Outside the cranium lie the proximal jugular and the slightly more distal nodose ganglions, which harbor the neuronal cell bodies of the sensory neurons [3, 4]. Nodose ganglion of adult male or female rats contains about 6000 neurons. The size of nodose neurons ranges from 15 to 50 μm in diameter [5].

Several markers for metabolic activity have been demonstrated in primary sensory neurons of vagus nerves — cytochrome oxidase, c-Fos and c-Jun proteins. The c-fos and c-jun belong to the family of the immediate early genes. They are expressed rapidly in the nodose ganglions and in the CNS after various forms of stimulation and their protein products c-Fos and c-Jun participate as “third messengers” in the regulation of neurotransmitter and peptide expression in those neurological structures [6]. Increase in c-Fos expression is now widely accepted as a marker of neuronal activity.

Central terminals of primary vagal afferents are mainly found in the nucleus tractus solitarius (NTS) and the area postrema, fewer in the dorsal motor nucleus of the vagus and in the trigeminal islands [3, 4, 7]. Afferent vagal fibres convey sensory information from the upper gastrointestinal tract to the CNS. In vagus nerve, afferent fibres outnumber efferent fibres by 10 to 1 [7]. Considering this, peripheral vagus nerve stimulation could be sufficiently effective to influence the central nervous system neurons activity.

Vagus nerve stimulation (VNS) has been introduced in therapy of some neurological disorders, as epilepsy [8], depression [9], various anxiety disorders [9], Alzheimer’s disease, migraines [10], fibromyalgia [11] and tinnitus [12]. It has also been reported, that stimulation of the vagus nerve influences cytokine production and improves survival in experimental sepsis, hemorrhagic shock and ischemia-reperfusion injury models [13]. Unexpectedly, in some patients treated with VNS therapy, weight decrease and reduction in food intake were observed. This fact has inspired researchers to investigate the mechanisms of weight loss after vagus nerve stimulation and to introduce VNS as a new method for obesity treatment [14, 15].

We previously showed that both short- and long-term vagus stimulation affect food intake and decrease body weight in rats [16, 17, 18, 19]. Moreover, Bugajski et al. [20] reported a decrease in meal size, a body weight reduction

and a decrease in epididymal fat pad weight in obese rats. Ziomber et al. [21] presented, that modulation of left vagus nerve by solenoid placed in rats with magnetic field exposure leads to decrease in body weight gain in growing animals. We hypothesized that peripheral electrical vagus nerve stimulation can "mimic" satiety signals from the gut to the CNS, leading to body mass reduction and to decrease in food consumption. To confirm our theory we assessed in this study the morphological properties and c-Fos immunoreactivity in neurons of nodose ganglion of vagus nerve after chronic left vagus nerve stimulation. We have stimulated left vagus nerve peripherally (the sub-diaphragmatic part), to limit possible side effects of VNS on the heart or the lungs, as the right vagus feeds those organs. Nevertheless, we examined both — left and right nodose ganglion neurons.

MATERIALS AND METHODS

Male Wistar rats (n = 24) weighing at the beginning of the study 200 ± 25 g were used. The animals were fed the high fat diet to induce obesity (DIO) (Bento Kronen Products, Belgium) during whole experiment. The caloric distribution of the DIO was: protein 29.5%, fat 45.6%, carbohydrates 24.9%, and metabolizable energy was 4.34 kcal/g. All animals were housed in the same optimal conditions of the lifestyle with food and water ad libitum and at 23 ± 2°C temperature on a 12 : 12-hour dark/light cycle. The Jagiellonian University Bioethical Committee approved the care and use of the animals.

After about 2 weeks of adaptation to new environmental conditions and fat diet rats were starved for 12 hours and operated in general anesthesia induced with sodium pentobarbital given intraperitoneally (Vetbutal, 0.25 mg/kg, i.p., Biowet, Puławy, Poland). The rats were randomly divided into two groups: 1. rats with active microstimulator (MS) connected by electrodes with the left vagal nerve (MS group, n = 12), 2. animals with inactive MS without electrodes on the vagal nerve (control group, n = 12). The MS for chronic vagal stimulation (Institute of Electron Technology, Cracow, Poland) was placed into the subcutaneous pocket and the ends of the MS silver electrodes were wrapped around the subdiaphragmatic left vagal nerve; cathode and anode were positioned at 0.5 cm distance. The parameters of the impulses generated by MS were: unipolar rectangular pulses duration — 10 ms, amplitude — 200 mV, frequency — 0.05 Hz. The parameters of MS stimulation were set, based on our previous studies [16, 17]. In the second group of animals inactive MS was implanted and laparotomy was performed (control group).

After 3 weeks of MS stimulation, randomly chosen rats from MS group (n = 6) and control group (n = 6) were euthanized and nodose ganglions (left and right) were taken for analysis. The other rats were stimulated until the

end of the third month, then they were euthanized and further analyses were made. Nodose ganglions were formalin fixed, paraffin-embedded block were made, and 5 μm slices were cut and mounted on silanized slides for further stainings. Firstly, specimens were hematoxylin — eosin stained for routine histology. For immunostaining heat induced antigen retrieval was carried out to unmask antigen determinants (20 min. in 95°C Target Retrival Solution — DAKO). Nodose ganglion neurons were identified using primary polyclonal antibodies (rabbit PGP 9.5 — DAKO; 1 : 200, 24 h at 4°C) with LSAB2 (DAKO) visualization system using diamino-benzidine — DAB — (DAKO) as a chromogen, according to protocol provided by manufacturer. c-Fos positive neurons were indentified with immunofluorescence (IF) method. After incubation with primary rabbit polyclonal anti-c-Fos antibody with 0.5% Triton X-100 (anti c-Fos, Santa Cruz Biotechnology; 1 : 100, 24 h at 4°C), secondary biotynylated antibody goat anti-rabbit (Jackson ImmunoResearch Laboratories, 1 : 800) was added for 2 h at room temperature. After rinsing in phosphate buffer anti-goat antibody conjugated with Cy3 (Jackson ImmunoResearch Laboratories, Inc., 1 : 800) was applied for 1 h. Slides were mounted with Fluorescent Mounting Medium (DAKO Cytomation) and analysis of specimens were performed under fluorescence JENAMED2 (Zeiss) microscope with wave length 570 nm.

The area of PGP 9.5 positive cells was assessed by image analysis (Multiscan 18.03, CSS) in light microscopy. In each specimen at least 200 neurons were analyzed. Cells positive for c-Fos staining were counted and data were expressed as a percentage of total neurons in nodose ganglion in IF stained specimens. Data are expressed as mean and standard deviation (SD). Results were analyzed by one-way analysis of variance (ANOVA), followed by post-hoc LSD test with STATISTICA 9.0 software package (StatSoft, Tulsa). Statistical significance was set at $p < 0.05$.

RESULTS

Chronic vagus nerve stimulation increased percentage of c-Fos positive neurons in the left nodose ganglions in both, 3-weeks and 3-months stimulated rats. Assessment of c-Fos positive cells showed significant ($p = 0.00006$) increase in percentage of those neurons in the left nodose ganglion ($4.19 \pm 2.26\%$) compared to control ($1.44 \pm 1.18\%$) in 3-weeks experiment. Interestingly, there was also an increase in c-For neurons in the right nodose ganglion ($2.64 \pm 1.98\%$) compared to control ($1.50 \pm 1.53\%$, $p = 0.054$) (Fig. 1).

Data obtained from 3-month experiment were similar: $4.97 \pm 1.54\%$; for left VNS and $1.68 \pm 1.51\%$ for left control nodose ganglion ($p = 0.0001$), and ($2.66 \pm 1.88\%$ versus $1.45 \pm 1.57\%$, $p = 0.024$) for the right VNS and control respectively (Fig. 2).

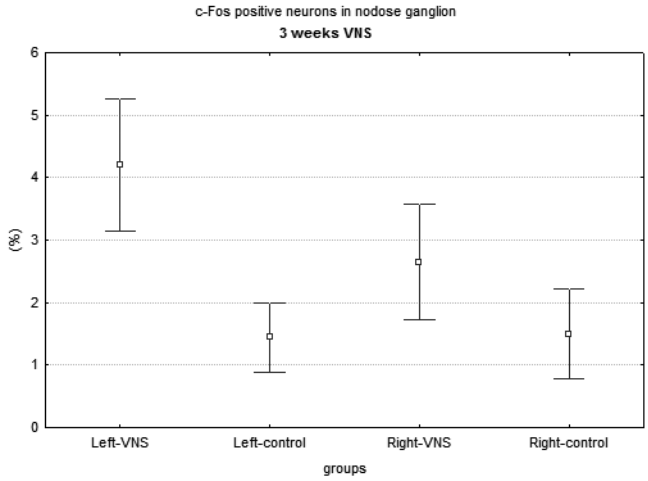


Fig. 1. c-Fos positive neurons percentage in the rats after 3 weeks of electrical left vagus nerve stimulation (VNS) and control in the left and right nodose ganglions. Data expressed as mean ± 0.95 confidence interval

Ryc. 1. Odsetek neuronów c-Fos pozytywnych w lewym i prawym zwoju guzowatym szczura po 3 tygodniach elektrycznej stymulacji nerwu błędnego oraz w grupie kontrolnej. Dane przedstawiono jako średnią wraz z przedziałem ufności 0,95

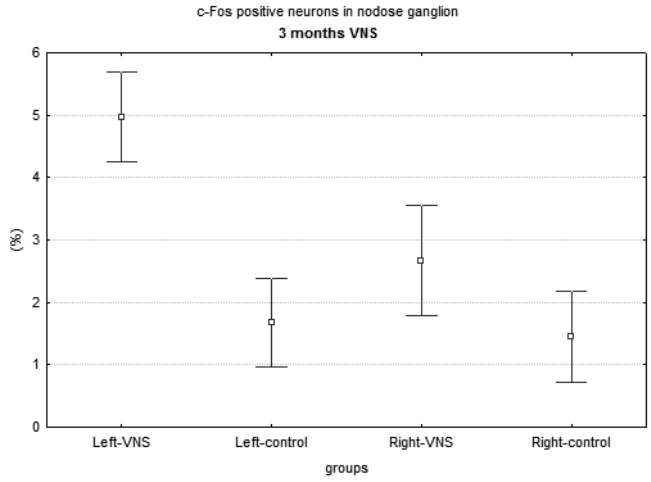


Fig. 2. c-Fos positive neurons percentage in the rats after 3 months of electrical left vagus nerve stimulation (VNS) and control in the left and right nodose ganglions. Data expressed as mean ± 0.95 confidence interval

Ryc. 2. Odsetek neuronów c-Fos pozytywnych w lewym i prawym zwoju guzowatym szczura po 3 miesiącach elektrycznej stymulacji nerwu błędnego oraz w grupie kontrolnej. Dane przedstawiono jako średnią wraz z przedziałem ufności 0,95

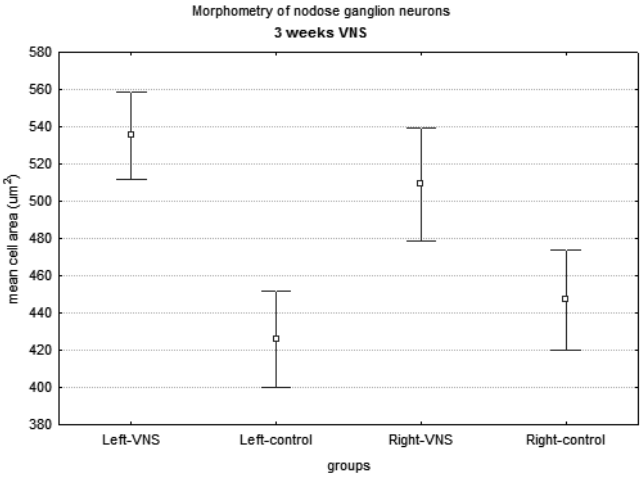


Fig. 3. Morphometrical assessment of the left and right nodose ganglion neurons in the rats after 3 weeks of electrical left vagus nerve stimulation (VNS) and control groups. Data expressed as mean ± 0.95 confidence interval

Ryc. 3. Analiza morfometryczna neuronów w lewym i prawym zwoju guzowatym szczura po 3 tygodniach elektrycznej stymulacji nerwu błędnego oraz w grupie kontrolnej. Dane przedstawiono jako średnią wraz z przedziałem ufności 0,95

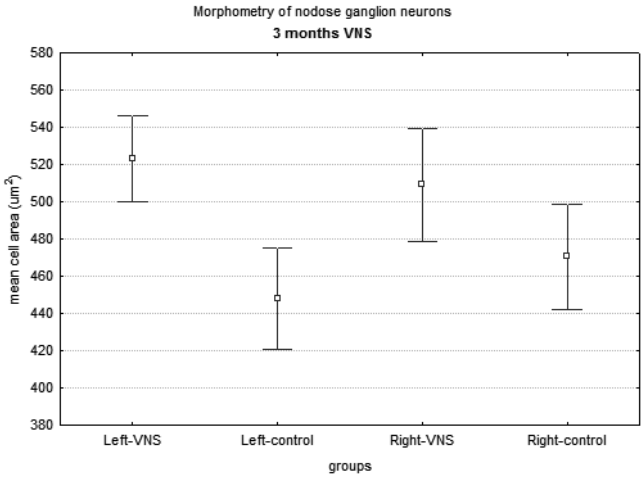


Fig. 4. Morphometrical assessment of the left and right nodose ganglion neurons in the rats after 3 months of electrical left vagus nerve stimulation (VNS) and control groups. Data expressed as mean ± 0.95 confidence interval

Ryc. 4. Analiza morfometryczna neuronów w lewym i prawym zwoju guzowatym szczura po 3 miesiącach elektrycznej stymulacji nerwu błędnego oraz w grupie kontrolnej. Dane przedstawiono jako średnią wraz z przedziałem ufności 0,95

There were no significant differences in c-Fos count between 3 weeks and 3 months stimulated animals, respectively. In both sets of experiments c-Fos positive neurons percentage did not differ between left and right nodose ganglions in controls group.

Image analysis of nodose ganglion neurons showed significant ($p = 0.001$) increase in mean cell area in rats with VNS in the left nodose ganglion (535.2 ± 172.8 μm^2) compared to control value (425.7 ± 163.6 μm^2) after 3 weeks of stimulation. Surprisingly, there was also a small increase in right nodose ganglion area (509.1 ± 198.8 μm^2 versus control 446.9 ± 171.8 μm^2) after 3 weeks of stimulation, but not significant ($p > 0.05$) (Fig. 3).

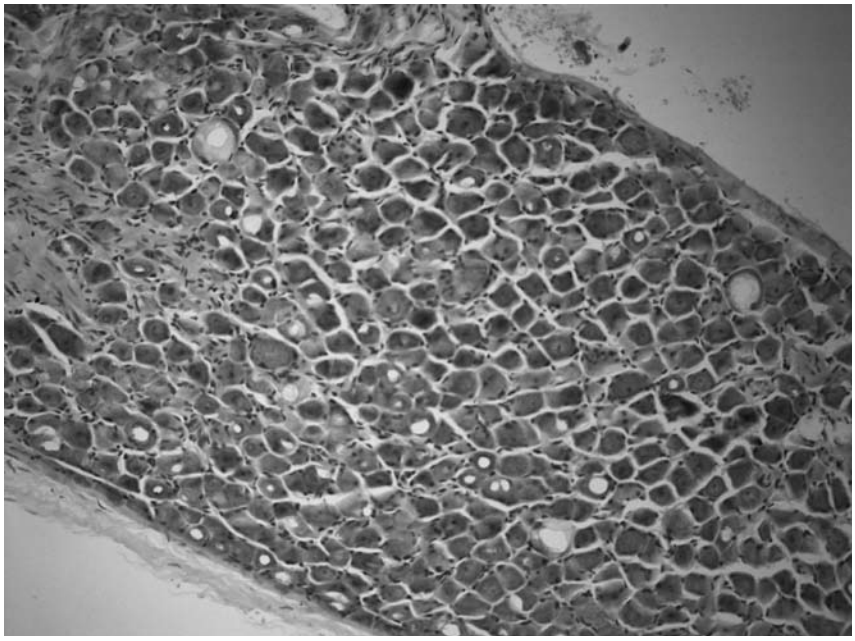
Data obtained from 3-month experiment were similar: an increase in mean neurons area in rats with VNS in the left nodose ganglion as well as in the right were present: for the left nodose ganglion neurons (523.2 ± 168.9 μm^2 for left VNS versus 448.1 ± 172.2 μm^2 for control, significant; $p = 0.0001$) and for the right (509.1 ± 198.7 μm^2 for right VNS versus 470.5 ± 180.8 μm^2 for control respectively, non significant; $p > 0.05$) (Fig. 4).

There were no significant differences between 3-weeks and 3-months experimental groups. In both experimental groups neuron area values were slightly higher, but not significantly, for the right vagal nodose ganglion neurons in controls compared to the left (about 5%). Nodose ganglion of control rat in routine hematoxylin-eosin staining and PGP 9.5 positively — stained cells are shown in Figure 5.

DISCUSSION

The cell bodies of vagal visceral afferents are contained within the nodose ganglia. Central projections of these neurons enter the brain stem and make synaptic connection with second order neurons that distribute visceral information throughout central neuronal structures [1, 7]. The branching pattern of vagus nerve in the abdomen has been best characterized in the rat. The ventral or anterior trunk, which is contiguous with the left cervical vagus, branches into the common hepatic, ventral gastric and ventral (or accessory) celiac branches. The common hepatic branch divides typically at about one third the distance between diaphragm and gastric cardia. Using electron microscopy on cross sectioned nerves, and supra- vs. infra-nodose cervical, thoracic, or abdominal vagotomies, the proportion of myelinated and unmyelinated axons as well as afferent, efferent and adventitial axons have been estimated. In the rat there are about 11,000 nerve fibers in each of the sub diaphragmatic trunks, of which about 8000 are afferent and 3000 efferent. Less than 1% of all fibers are myelinated, and the average size of the unmyelinated fibers is about 0.8 mm, with some axons as thin as 0.1 mm [4, 22]. As we desired to replace

A



B

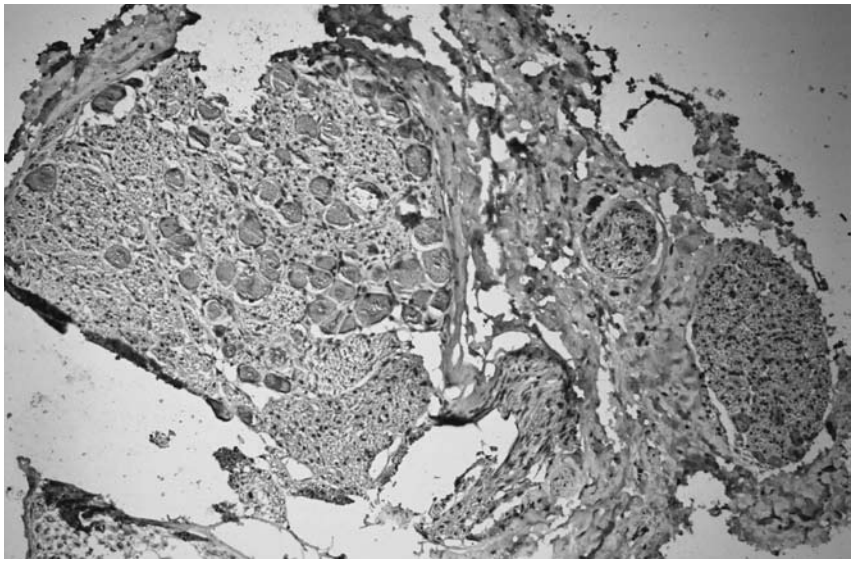


Fig. 5. Nodose ganglion of the control rat. (A) Hematoxylin-eosin staining, neurons stained pink. Magnification 200 ×. (B) PGP9.5/DAB staining, nodose ganglion neurons stained brown. Magnification 200 ×

Ryc. 5. Zwój guzowaty szczura z grupy kontrolnej. (A) Barwienie hematoksylina-eozyna. Neurony wybarwione na różowo. Powiększenie 200 ×. (B) Barwienie PGP9.5/DAB. Neurony wybarwione na brązowo. Powiększenie 200 ×

the physiological satiety input from the gut into the CNS by VNS, such subdiaphragmatic stimulation of the left vagus nerve has strong anatomical and physiological background.

We use VNS in rats as a study method of obesity mechanisms, firstly — we have to establish the morphological criterion of VNS to be sufficiently effective, and secondly — to prove, that VNS, even after few weeks of stimulation still exerts its effects on vagal afferents neurons. In this study we have chosen 3-week and 3-month periods, as we do not carry the VNS longer.

In current study we examined effects of the left vagus nerve stimulation on nodose ganglion neurons. When the left and right vagal trunks feed different parts of the gastrointestinal tract and contribution of both is important, the decision, which one of trunks should be stimulated, remains controversial. Previous works from our laboratory [16, 17] demonstrated that bilateral VNS seems to be more effective than unilateral stimulation, but the others proved that unilateral stimulation could be effective as well. To limit possible side effects on the heart or the lungs, we decided to apply a chronic VNS by microstimulator placed on the left vagus nerve. Electrodes were put close to the gastro-esophageal junction to stimulate the small unmyelinated C fibres and to avoid stimulation of fibres that join the trunk from the heart and lungs, as discussed by Val-Laillet [23]. We used constant voltage microstimulator implanted for at least 3 months, and set up higher frequency of stimulation similar to our previous experiments.

Assessment of c-Fos expression as a marker of neural activity offers several advantages. c-Fos is a delayed marker of brain activation. The neuronal process that mediates neuronal activity and behavior at the time of stimulation may also initiate the slower processes of immediate-early gene activation and protein synthesis. Activation of the c-Fos gene by a sensory stimulus results in c-Fos protein synthesis within 1 h [24]. Because c-Fos is visualized about 1 h after stimulation, the presence of c-Fos in stimulated neural cells will be still detectable, even the stimulus were withdrawn. The pattern of c-Fos expression in the nodose ganglion provides cellular resolution of neural activity that can be quantified by counting the number of labeled cells [25]. The degree of c-Fos expression can then be even correlated with stimulus magnitude. Finally, c-Fos assessment allows mapping of neuronal populations activated by a stimulus. The central processing of visceral sensation is mediated by many nuclei throughout the brain. Because many sections can be processed for c-Fos, activity in multiple brain regions of the same animal can be visualized for the analysis of a distributed network. Moreover, the patterns of c-Fos expression can be interpreted against a large database of c-Fos references.

First results on c-Fos after vagus nerve stimulation were disappointing — the c-Fos were not found in the nodose ganglion following either electrical stimulation or deafferentation of the cervical vagus nerve [26]. Later studies

showed that c-Fos has a low basal level of expression in the nodose ganglion but is greatly increased by crush or axotomy of the cervical vagus nerve. Currently, it is well established that c-Fos expressed neurons are present in nodose ganglia after variety of stimuli [27–36].

The amount of c-Fos positive, non-stimulated neurons in nodose ganglion do not exceed about 1–2% of cells. Our results are consistent with those data. We have found c-Fos neurons count to be less than 2% in control groups, both in the left and right nodose ganglions. However, after VNS almost four-fold increase in c-Fos positive cells was observed.

The results of our study indicate that VNS can lead to immunohistochemical as well as morphological changes of nodose ganglia neurons. It is important to mention, that morphological changes in the left, and partially in the right nodose neurons were noticed. There was a major increase in the area of neurons in VNS animals in the left nodose ganglion. The increase in size of neurons — "neuronal hypertrophy" — could be explained as a mode of adaptation to the increased activity of their targeted tissue, as a structural response to the functional overload imposed by VNS. Similar changes have been mentioned by Kosta in nodose ganglion neurons after intensive exercises in rats. The physical exercise in her study did not exert any influence on either ganglion size or total number of neurons, but the mean perikaryal volume of neurons significantly increased in trained animals [37]. Authors have speculated that since the nodose ganglia neurons showed extremely high potential for neurogenesis [38], so there is a small possibility that exercise causes neurogenesis in rat nodose ganglions. Since we reported similar observation of nodose ganglions neurons, further investigation with indicators of cell proliferation would be extremely helpful to obtain decisive results.

The sensory cells of the nodose and jugular ganglia of the rat have been also quantitatively evaluated in longitudinal paraffin sections obtained from rats after neonatal capsaicin treatment by Carobi [39]. According to this report, the right vagal ganglions contain significantly more neurons than the left one, particularly neurons with somata having sectioned areas 200–400 μm^2 and longest diameters of 15–25 μm. It is consistent with our data, as we showed an increase in the mean right nodose ganglion neurons area compared to the left one. Neonatal capsaicin treatment reduced the number of neurons in both the left and right ganglia to about 30% of control values and destroyed neurons with sectioned areas of 100–600 μm^2 and longest diameters of 15–35 μm, but had no statistically significant effects on larger neurons. It is possible, that VNS in our model somehow influenced smaller neurons, or just decreased PGP 9.5 expression, leading to decrease in the cell area.

Vagal nerve stimulation caused significant changes in the left nodose ganglion (which were largely expected), but, surprisingly, some changes in c-Fos neuron count and the cell area of the right nodose ganglion cells were also

present. It is difficult to clarify such observation. It has been reported that in some species, including rats, thoracic and abdominal communicating branches between the left and right trunks exist, with afferent and/or efferent axons from one side crossing over to the other side [22, 40]. However, in the rat, there is little evidence from retrograde experiments for crossing efferent axons, but as many as 20% of afferent axons may cross at the thoracic level [41]. In the ferret an abdominal communicating branch has been characterized, with both efferent and afferent axons crossing sides [40]. Having been given these facts, there is still a possibility which cannot be excluded that VNS in current model evoked weak response in the right nodose ganglion. We can also speculate, whether VNS by some efferent mechanisms may induce cellular response, which activates vagal afferents, for example by gastric mast cells [19]. That phenomenon needs to be further examined.

CONCLUSIONS

Electrical vagus nerve stimulation with microstimulator placed on the left vagal nerve causes changes in neurons area and c-Fos expression in nodose ganglions, mainly in the left, but also in the right nodose ganglion. Those changes observed in both — left and right vagal ganglions after VNS are present during the whole three-month experiment. Increase in c-Fos expression in nodose ganglion neurons confirms the afferent transmission of the signal (generated by MS) from periphery to the brain by the vagus nerves, and allows implementing the VNS technique as a new, effective method in obesity mechanisms research and management.

Krzysztof Gil, Andrzej Bugajski, Beata Skowron, Piotr Thor

STYMULACJA NERWU BŁĘDNEGO ZWIĘKSZA EKSPRESJĘ C-FOS W ZWOJACH GUZOWATYCH NERWU BŁĘDNEGO SZCZURA

Streszczenie

Centralny układ nerwowy otrzymuje informacje z przewodu pokarmowego oraz dostosowuje pobieranie pokarmu głównie dzięki informacjom docierającym za pośrednictwem nerwów błędnych. Nasze dotychczasowe badania wykazały, że przewlekła elektryczna stymulacja nerwu błędnego imitująca sygnały sytości wysyłane z przewodu pokarmowego powoduje spadek masy ciała oraz zmniejszone pobieranie pokarmu u szczurów.

Celem przeprowadzonych doświadczeń było zbadanie wpływu przewlekłej stymulacji nerwu błędnego na neurony zwojów guzowatych nerwu błędnego szczura oceniane poprzez analizę ekspresji białka c-Fos oraz metodami morfometrycznymi.

Szczurom rasy Wistar (n = 24) wszczepiono mikrostymulatory (MS) oraz karmiono dietą wysokokaloryczną przez 3 miesiące. Lewy nerw błędny stymulowano elektrycznie (impulsy prostokątne,

czas trwania 10 ms, amplituda 200 mV, częstotliwość 0,05 Hz). 12 szczurów (6 — kontrola oraz 6 — z MS) badano odpowiednio po 3 tygodniach oraz po 3 miesiącach. Pobierano lewy i prawy zwój guzowaty nerwu błędnego, utrwalano w formalinie i wykonywano preparaty techniką parafinową. Neurony identyfikowano metodą immunohistochemiczną (PGP 9,5 jako marker) oraz liczono odsetek neuronów wykazujących pozytywny odczyn z przeciwciałem anty c-Fos.

Po 3 tygodniach stymulacji odsetek neuronów c-Fos pozytywnych w zwojach guzowatych był podwyższony znamiennie w lewym (4,19%) oraz słabo w prawym zwoju (2,64%) w odniesieniu do kontroli (1,44%). Podobne dane uzyskano po 3 miesiącach stymulacji: 4,97%; 2,66% i 1,68%, odpowiednio dla zwoju lewego i prawego po stymulacji oraz kontroli. Nie stwierdzono różnic pomiędzy odpowiednimi grupami po 3-tygodniowej i 3-miesięcznej stymulacji.

Podwyższony odsetek neuronów c-Fos pozytywnych w lewym zwoju guzowatym nerwu błędnego potwierdza aferentną transmisję sygnału generowanego przez mikrostymulator z obwodu do mózgu drogą nerwu błędnego.

REFERENCES

1. *Morton G.J.*, *Cummings D.E.*, *Baskin D.G.*, *Barsh G.S.*, *Schwartz M.W.*: Central nervous system control of food intake and body weight. Nature 2006; 443: 289–295. **— 2.** *Schwartz M.W.*, *Woods S.C.*, *Porte D., Jr.*, *Seeley R.J.*, *Baskin D.G.*: Gentral nervous system control of food intake. Nature 2000; 404: 661–671. **— 3.** *Paxinos G.* (ed.): The Rat Nervous System, Third Edition. Elsevier Academic Press, 2004. **— 4.** *Berthoud H.R.*, *Neuhuber W.L.*: Functional and chemical anatomy of the afferent vagal system. Auton Neurosci. 2000 Dec 20; 85(1–3): 1–17. **— 5.** *Zhuo H.*, *Ichikawa H.*, *Helke C.J.*: Neurochemistry of the nodose ganglion. Prog Neurobiol. 1997 Jun; 52(2): 79–107. **— 6.** *Morgan J.I.*, *Curran T.*: Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. Annu Rev Neurosci. 1991; 14: 421–451. **— 7.** *Grundy D.*: Neuroanatomy of visceral nociception: vagal and splanchnic afferent. Gut. 2002 Jul; 51 Suppl 1: i2–5. **— 8.** *Noe K.H.*, *Locke D.E.*, *Sirven J.I.*: Treatment of Depression in Patients with Epilepsy. Curr Treat Options Neurol 2011. **— 9.** *Groves D.A.*, *Brown V.J.*: Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. Neurosci Biobehav Rev 2005; 29: 493–500. **— 10.** *Cecchini A.P.*, *Mea E.*, *Tullo V.*, *Curone M.*, *Franzini A.*, *Broggi G.*, *Savino M.*, *Bussone G.*, *Leone M.*: Vagus nerve stimulation in drug-resistant daily chronic migraine with depression: preliminary data. Neurol Sci 2009; 30 Suppl 1: S101–S104.

11. *Finocchi C.*, *Villani V.*, *Casucci G.*: Therapeutic strategies in migraine patients with mood and anxiety disorders: clinical evidence. Neurol Sci 2010; 31 Suppl 1: S95–S98. **— 12.** *Engineer N.D.*, *Riley J.R.*, *Seale J.D.*, *Vrana W.A.*, *Shetake J.A.*, *Sudanagunta S.P.*, *Borland M.S.*, *Kilgard M.P.*: Reversing pathological neural activity using targeted plasticity. Nature 2011; 470: 101–104. **— 13.** *Johnston G.R.*, *Webster N.R.*: Cytokines and the immunomodulatory function of the vagus nerve. Br J Anaesth 2009; 102: 453–462. **— 14.** *Camilleri M.*, *Toouli J.*, *Herrera M.F.*, *Kow L.*, *Pantoja J.P.*, *Billington C.J.*, *Tweden K.S.*, *Wilson R.R.*, *Moody F.G.*: Selection of electrical algorithms to treat obesity with intermittent vagal block using an implantable medical device. Surg Obes Relat Dis 2009; 5: 224–229. **— 15.** *Camilleri M.*, *Toouli J.*, *Herrera M.F.*, *Kulseng B.*, *Kow L.*, *Pantoja J.P.*, *Marvik R.*, *Johnsen G.*, *Billington C.J.*, *Moody F.G.*, *Knudson M.B.*, *Tweden K.S.*, *Vollmer M.*, *Wilson R.R.*, *Anvari M.*: Intra-abdominal vagal blocking (VBLOC therapy): clinical results with a new implantable medical device. Surgery 2008; 143: 723–731. **— 16.** *Krolczyk G.*, *Zurowski D.*, *Sobocki J.*, *Slowiaczek M.P.*, *Laskiewicz J.*, *Matyja A.*, *Zaraska K.*, *Zaraska W.*, *Thor P.J.*: Effects of continuous microchip (MC) vagal neuromodulation on gastrointestinal function in rats. J Physiol Pharmacol 2001; 52: 705–715. **— 17.** *Laskiewicz J.*, *Krolczyk G.*, *Zurowski D.*,

Enck P., *Thor P.J.*: Capasaicin induced deafferentation enhances the effect of electrical vagal nerve stimulation on food intake and body mass. J Physiol Pharmacol 2004; 55: 155–163. — **18.** *Laskiewicz J.*, *Krolczyk G.*, *Zurowski G.*, *Sobocki J.*, *Matyja A.*, *Thor P.J.*: Effects of vagal neuromodulation and vagotomy on control of food intake and body weight in rats. J Physiol Pharmacol 2003; 54: 603–610. — **19.** *Gil K.*, *Bugajski A.*, *Kurnik M.*, *Zaraska W.*, *Thor P.*: Physiological and morphological effects of long-term vagal stimulation in diet induced obesity in rats. J Physiol Pharmacol 2009; 60 Suppl 3: 61–66. — **20.** *Bugajski A.J.*, *Gil K.*, *Ziomber A.*, *Zurowski D.*, *Zaraska W.*, *Thor P.J.*: Effect of long-term vagal stimulation on food intake and body weight during diet induced obesity in rats. J Physiol Pharmacol 2007; 58 Suppl 1: 5–12.

21. *Ziomber A.*, *Juszczak K.*, *Kaszuba-Zwoinska J.*, *Machowska A.*, *Zaraska K.*, *Gil K.*, *Thor P.*: Magnetically induced vagus nerve stimulation and feeding behavior in rats. J Physiol Pharmacol 2009; 60: 71–77. — **22.** *Prechtl J.C.*, *Powley T.L.*: The fiber composition of the abdominal vagus of the rat. Anat Embryol (Berl). 1990; 181(2): 101–115. — **23.** *Val-Laillet D.*, *Biraben A.*, *Randuineau G.*, *Malbert C.H.*: Chronic vagus nerve stimulation decreased weight gain, food consumption and sweet craving in adult obese minipigs. Appetite 2010; 55: 245–252. — **24.** *Morgan J.I.*, *Curran T.*: Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. Annu Rev Neurosci. 1991; 14: 421–451. — **25.** *Sagar S.M.*, *Sharp F.R.*, *Curran T.*: Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science. 1988 Jun 3; 240(4857): 1328–1331. — **26.** *Herdegen T.*, *Kummer W.*, *Fiallos C.E.*, *Leah J.*, *Bravo R.*: Expression of c-JUN, JUN B and JUN D proteins in rat nervous system following transection of vagus nerve and cervical sympathetic trunk. Neuroscience. 1991; 45(2): 413–422. — **27.** *Miller A.D.*, *Ruggiero D.A.*: Emetic reflex arc revealed by expression of the immediate-early gene c-fos in the cat. J Neurosci. 1994 Feb; 14(2): 871–888. — **28.** *Berthoud H.R.*, *Patterson L.M.*, *Willing A.E.*, *Mueller K.*, *Neuhuber W.L.*: Capsaicin-resistant vagal afferent fibers in the rat gastrointestinal tract: anatomical identification and functional integrity. Brain Res. 1997 Jan 23; 746(1–2): 195–206. — **29.** *Goehler L.E.*, *Gaykema R.P.*, *Hammack S.E.*, *Maier S.F.*, *Watkins L.R.*: Interleukin-1 induces c-Fos immunoreactivity in primary afferent neurons of the vagus nerve. Brain Res. 1998 Sep; 804(2): 306–310. — **30.** *Groneberg D.A.*, *Wiegand S.*, *Dinh Q.T.*, *Peiser C.*, *Springer J.*, *Fischer A.*: Expression of immediate early genes in sensory ganglia. Neurochem Res. 2001 Oct; 26(10): 1113–1117.

31. *Wu X.Y.*, *Zhu J.X.*, *Gao J.*, *Owyang C.*, *Li Y.*: Neurochemical phenotype of vagal afferent neurons activated to express C-FOS in response to luminal stimulation in the rat. Neuroscience. 2005; 30(3): 757–767. — **32.** *Koda S.*, *Date Y.*, *Murakami N.*, *Shimbara T.*, *Hanada T.*, *Toshinai K.*, *Niijima A.*, *Furuya M.*, *Inomata N.*, *Osuye K.*, *Nakazato M.*: The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. Endocrinology. 2005 May; 146(5): 2369–2375. — **33.** *Li Y.*, *Wu X.*, *Yao H.*, *Owyang C.*: Secretin activates vagal primary afferent neurons in the rat: evidence from electrophysiological and immunohistochemical studies. Am J Physiol Gastrointest Liver Physiol. 2005 ct; 289(4): G745–752. — **34.** *Covasa M.*, *Ritter R.C.*: Reduced CCK-induced Fos expression in the hindbrain, nodose ganglia, and enteric neurons of rats lacking CCK-1 receptors. Brain Res. 2005 Jul 27; 1051(1–2): 155–163. — **35.** *Li Y.*, *Wu X.*, *Zhou S.*, *Owyang C.*: Low-affinity CCK-A receptors are coexpressed with leptin receptors in rat nodose ganglia: implications for leptin as a regulator of short-term satiety. Am J Physiol Gastrointest Liver Physiol. 2011 Feb; 300(2): G217–227. — **36.** *Baraboi E.D.*, *St-Pierre D.H.*, *Shooner J.*, *Timofeeva E.*, *Richard D.*: Brain activation following peripheral administration of the GLP-1 receptor agonist exendin-4. Am J Physiol Regul Integr Comp Physiol. 2011 Oct; 301(4): R1011–1024. — **37.** *Košta V.*, *Guić M.M.*, *Aljinović J.*, *Carić A.*, *Grković I.*: The influence of exercise on morphological and neurochemical properties of neurons in rat nodose ganglia. Neurosci Lett. 2011 Feb 18; 490(1): 36–40. — **38.** *Czaja K.*, *Burns G.A.*, *Ritter R.C.*: Capsaicin-induced neuronal death and proliferation of the primary sensory neurons located in the nodose ganglia of adult rats. Neuroscience. 2008 Jun 23; 154(2): 621–630. — **39.** *Carobi C.*: A quantitative investigation of the effects of neonatal capsaicin treatment on vagal afferent neurons in the rat. Cell Tissue Res. 1996 Feb; 283(2): 305–311.

— 40. *Fitzakerley J.L.*, *Lucier G.E.*: Connections of a vagal communicating branch in the ferret. I. Pathways and cell body location. Brain Res Bull. 1988 Feb; 20(2): 189–196.

41. *Sauter J.F.*, *Niijima A.*, *Berthoud H.R.*, *Jeanrenaud B.*: Vagal neurons and pathways to the rat's lower viscera: an electrophysiological study. Brain Res Bull. 1983 Nov; 11(5): 487–491.

Department of Pathophysiology
Jagiellonian University Medical College
Kraków, Poland

Address for correspondence:
Krzysztof Gil MD, Ph.D.
Department of Pathophysiology,
Jagiellonian University Medical College
ul. Czysta 18, 31-121 Kraków, Poland
Phone: +48 12 633 39 47, fax: +48 12 632 90 56
e-mail: mpgil@cyf-kr.edu.pl