ORIGINAL ARTICLE

Electrical chronic vagus nerve stimulation activates the hypothalamic-pituitary-adrenal axis in rats fed high-fat diet

Krzysztof Gil, Andrzej Bugajski, Magdalena Kurnik, Piotr Thor

Department of Pathophysiology, Jagiellonian University Medical College, Krakow, Poland

Correspondence to: Krzysztof Gil, MD., PhD.
Department of Pathophysiology, Jagiellonian University – Medical College
Czysta 18 Street, 31-121 Krakow, Poland.
TEL: +4812 6333947; FAX: +4812 6329056; E-MAIL: mpgil@cyf-kr.edu.pl

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Abstract

OBJECTIVES: The brain and the gut communicate bi-directionally through the brain–gut axis. The key role in such interactions plays autonomic nervous system and its major component, the vagus nerve. There is growing evidence that vagus nerve stimulation (VNS) has a suppressive effect on both short- and long-term feeding in animal models. In the present study, we investigated the effect of VNS on the hypothalamic pituitary adrenal axis, feeding behavior and appetite in rats fed a high-fat diet.

METHODS: Adult male Wistar rats were implanted with a microstimulator (MS) and fed a high-fat diet throughout the study (42 days). The left vagus nerve was stimulated subdiaphragmatically by electrical pulses (10 ms, 200 mV, 1 Hz or 10 Hz respectively, 12 h a day) generated by the MS. Daily food intake and body weight were measured. At the end of the experiment, animals were euthanized and serum corticosterone levels were assessed by immunoassays. Adipose tissue content was evaluated by measuring epididymal fat pads' weight. To determine whether VNS activated food-related areas of the brain, neuronal c-Fos induction in the nucleus of the solitary tract (NTS) was assessed.

RESULTS: Chronic VNS decreased food intake, body weight gain and epididymal fat pad weight in stimulated animals compared to control animals. Serum corticosterone concentrations were significantly elevated following VNS, and neuronal responses in the NTS were observed.

CONCLUSIONS: The study demonstrates that chronic electrical VNS exerts anorexigenic effects on food intake and body weight gain, and the hypothalamic pituitary adrenal axis activation may contribute to these effects.

INTRODUCTION

The brain and the gut communicate bi-directionally through the brain–gut axis. The key role in such interactions plays autonomic nervous system and its major component, the vagus nerve (VN). VN influences the neuro-endocrine-immune axis to maintain homeostasis through the activation of the hypothalamic pituitary adrenal (HPA) axis by its afferents (Jänig 2008). Stress factors transmitted *via* vagal afferents cause activation of parvocellular neurons of the paraventricular nucleus of the hypothalamus and the consequent release of the neuropeptides corticotropin releasing hormone (CRH)

and arginine vasopressin (AVP) into the hypophyseal portal system. CRH and AVP act on corticotrope cells of the anterior pituitary gland and stimulate secretion of peptides derived from pro-opiomelanocortin which include adrenocorticotropin (ACTH), the opioid peptide β-endorphin and α-melanocyte-stimulating hormone. ACTH acts on the cortex of the adrenal glands to stimulate the synthesis of glucocorticoids. In humans the predominant glucocorticoid is cortisol, whereas in rodents the key glucocorticoid is corticosterone. Glucocorticoids are the final effectors of the HPA axis and participate in the control of whole body homeostasis and the organism's response to stress. (Tilbrook & Clarke 2006; Tsigos & Chrousos 2002).

The vagus nerve is the longest of the cranial nerves and a main component of the parasympathetic nervous system providing innervation of several organs of the neck, thorax, and abdomen. The vagal trunk innervating the gut is composed mainly of unmyelinated fibers with afferent fibers being the major component (over 80%), while vagal efferents represent less than 20%. Vagal afferents conduct information to the brain from periphery, influencing feeding behavior and digestive reflexes. Vagal efferents originated from neurons located in brainstem nuclei – the dorsal motor nucleus of the vagus (DMX) and nucleus ambiguus – control autonomic functions. The VN regulates heart rate, blood pressure, and gastrointestinal motility and secretion (Berthoud & Neuhuber 2000; Prechtl & Powley 1990). The right VN innervates the sinoatrial node (involved in the pacemaker function of the heart), and the left VN innervates the atrioventricular node (regulating the force of contraction of the heart muscle with less influence over heart rate). Vagus nerve stimulation (VNS) of the right nerve, compared to the left, caused a greater reduction in heart rate, whereas stimulation of the left VN had no effect on heart rate (Woodbury & Woodbury 1990). Thus, in experimental as well as clinical conditions VNS is performed mainly on the left vagus (Bonaz *et al.* 2013).

The VN afferent neurons are located in the nodose ganglions and transmit sensory information to the nucleus of the solitary tract (NTS). Nucleus of the solitary tract, the dorsal motor nucleus of the vagus nerve and the area postrema constitute the dorsal vagal complex (DVC), which provide the major integrative center for ANS. Consequently, vagus nerve afferents to the central nervous system have been examined as a possible target for new therapeutic strategies. VNS therapy has been successfully adopted for the treatment of drug-resistant epilepsy and depression (Ondicova *et al.* 2010; Groves & Brown 2005). It has also been under investigation for the management of various anxiety disorders (Groves & Brown 2005), Alzheimer's disease, migraines (Cecchini *et al.* 2009), fibromyalgia (Finocchi *et al.* 2010), and tinnitus (Engineer *et al.* 2011). Moreover, stimulation of the vagus nerve increases the production of anti-inflammatory cytokines and thus may lead to the increased rate of survival in experimental sepsis, hemorrhagic shock and ischemia–reperfusion injury models (Johnston & Webster 2009; Mihaylova *et al.* 2012; Hiraki *et al.* 2012). In such vagus nerve manipulations, it has been surprisingly discovered, that VNS therapy induces alterations in food intake and weight decrease. This fact has encouraged us to investigate the possible mechanisms of the weight loss after vagus nerve stimulation.

We presumed that VNS decreases food intake and body weight gain by "mimicking" satiety signals transmitted from the gut to the brain, leading to the activation of the hypothalamic neurons resulting in achieving the state of satiety. Because vagal afferents project to second order neurons in the nucleus of the solitary tract, stimuli elicited by electrical pulses could rapidly change neural activity in the NTS to initiate hormonal changes, including the hypothalamic-pituitary-adrenal axis activation. We adopted an animal obesity model using high-fat diet (HFD), as obesity induced by high-fat diet mimics obesity in humans, and is widely considered as an appropriate model for studying dietary obesity (Hariri & Thibault 2010).

The first aim of this study was to test the hypothesis, whether VNS increases the HPA activity, assessed by corticosterone levels measurements. Secondly, we examined the effects of VNS with low (1Hz) and high (10Hz) frequencies on corticosterone levels and behavioral parameters of the rats (food intake, body weight gain and fat accumulation). Finally, we examined c-Fos positive neurons count in NTS following chronic electrical vagus nerve stimulation. The c-fos belongs to the family of the immediate early genes and is expressed rapidly in the nodose ganglions and in the central nervous system after various forms of stimulation, and thus the increase in c-Fos expression is now widely accepted as a marker of neuronal activity (Gil *et al.* 2011b; Hoffman & Lyo 2002).

MATERIAL AND METHODS

The experiments were performed in two sets.

First experiment

Twenty-four male Wistar rats, housed in individual cages, were used in this experiment. All animals were fed a high-fat diet (caloric distribution of the diet: protein 25.1%, fat 38.8%, carbohydrates 36.1%, metabolizable energy 4.34 kcal/g; Bento Kronen Products, Belgium) during the whole experiment. The temperature was maintained at 23±2 °C and animals were placed on a 12:12 h dark/light cycle. Food and water were provided ad libitum. Jagiellonian University Bioethical Committee approved the care and use of the animals (protocol number – 36/2008).

After 2 weeks of adaptation to the environmental conditions and fat diet rats were starved for 12 hours and operated under general anesthesia induced with

sodium pentobarbital given intraperitoneally (Vetbutal, 0.25 mg/kg, Biowet, Pulawy, Poland).

The rats were randomly divided into following groups: 1. rats with active microstimulator (MS) connected by electrodes with the left vagal nerve (MS 1 Hz group, n=8); 2. animals with inactive MS without electrodes on the vagal nerve (sham 1 Hz group, n=8), and 3. intact rats – without MS and electrodes (control 1 Hz group, n=8). The control group of animals (intact rats) was included in the study to eliminate any effects of surgical procedures on examined parameters, while sham operated group served as the most important reference group for the assessment of food intake, body weight, epididymal fat pad weight and corticosterone levels.

The MS for vagus chronic stimulation (designed by Institute of Electron Technology, Krakow, Poland) was placed during the surgery 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. In the second group, laparotomy was performed and inactive MS was implanted (sham group), while in the control group (intact) no surgical manipulations were performed. Food was restored the next day. After a one-week recovery period rats were put into cages exposed to electromagnetic field and the stimulation started (day 1st) as described previously (Gil *et al.* 2011a). The animals from the MS and sham groups were placed individually into plastic cages and exposed to the magnetic field. The magnetic field served as an external source of current in the MS wires connected to the left vagus nerve. The parameters of the magnetic field were set experimentally, to match amplitude, duration and frequency of impulses used in our experiment. The third (control) group of rats was placed into cages outside the magnetic field.

The parameters of the impulses generated by MS were based on our previous studies and set as follows: unipolar rectangular pulses, duration 10 ms, amplitude 200 mV, frequency 1 Hz. The stimulation of animals started every day at 6 p.m. and lasted 12 h until 6 a.m. the next morning (dark phase stimulation).

Daily food intake and body weight were measured each morning during the entire study. The amount of daily food intake was determined by subtracting the amount of food remaining from the amount given 24 h before. All the rats were killed by decapitation and weighted at the end of the experiment (day 42nd). Both epididymal fat pads, located between the cauda epididymis and the distal extremity of the testis, were dissected from the animal and weighted.

Blood samples collected at the end of the experiment were taken into tubes filled with aprotinin (0.6 TIU per 1ml of blood; Sigma-Aldrich, USA), left for 30 minutes for clotting formation and after centrifugation 1500 × g, 20 min at 4 °C (Megafuge 1.0R, Heraeus Instruments) serum samples were collected and frozen at –80 °C until further analysis. Glucose and hepatic enzymes (aspartate and alanine transaminases) were measured with chemistry immune-analyzer Olympus AU 600. Corticosterone serum levels were quantified by enzyme immunoassay method (EIA) according to the protocol provided by manufacturer (IDS Limited, Fountain Hills, AZ, USA). ELx800 Absorbance Microplate Reader (Bio-Tek) was used for the absorbance detection.

Second experiment

Twenty-four animals were fed high-fat diet and maintained in the same condition as described for the first experiment, while additional eight rats were fed the standard diet (caloric distribution of the standard diet was: protein 26.7%, fat 7.9%, carbohydrates 65.4%, metabolizable energy 2.86 kcal/g; Labofeed, Poland). This group of animals (*standard* group; without MS and electrodes) was included in the study, to compare the effects of high-fat diet on rats. The schema of the second experiment was similar to the first one, with the exception of the frequency of vagus nerve stimulation, which was set at 10 Hz.

At the end of the second set of experiments, following decapitation, the whole brain was removed from the skull and placed in a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 h. Activated neurons of the nucleus of the solitary tract (NTS) were visualized by immunochemistry method, according to the methodology previously described (Gil *et al.* 2011a), using primary polyclonal rabbit antibodies against c-Fos (Santa-Cruz, sc-52, diluted 1:200), using diaminobenzidine as a chromogen. The brown-stained cells stained were considered positive and analyzed using an AXIOPHOT light microscope (Zeiss, Germany).

Statistical analysis

Data are expressed as mean and standard deviation (SD), except for the corticosterone (standard error of mean – SEM). Results were analyzed by one-way analysis of variance (ANOVA), followed by post-hoc LSD test with STATISTICA 8.0 software package (StatSoft, Tulsa). Statistical significance was set at $p<0.05$.

RESULTS

Food intake, body weight and epididymal fat pad weight

Food intake, body weight and epididymal fat pad weight are presented in Table 1 and Table 2.

Peripheral electrical stimulation of the left vagal nerve reduced the daily and total food intake in MS 1 Hz and MS 10 Hz groups compared to sham and control groups in both experiments and these differences were statistically significant. Moreover, VNS significantly reduced body weight gain in these groups as well. Fat pad weight reflecting total body fat content was significantly lower in both MS groups compared to relevant sham and control (intact) groups of rats (Table 1 and Table 2).

Tab. 1. Mean food intake, body weight and epididymal fat pad weight in MS left vagus nerve stimulation (1Hz), sham operated, and control (intact) rats.

First experiment	Sham	MS–1Hz	Control
Food intake during experiment (g)	1025±65	891±89*	1042±78
Initial body weight (g) – day 1	498.5±33.1	501.9±27.1	509.1±45.1
Final body weight (g) – day 42	673.5±38.4	641.7±31	687.5±44.5
Body weight gain (g)	166.2±21	136.9±8.9*	162.8±43.4
Body weight gain increment over initial body weight (%)	33.5	27.9*	32.3
Epididymal fat pad weight (g)	13.7±3.4	11.8±1.5*	14.7±4.7

Asterisks (*) indicate significant differences between MS–1Hz group and sham and control group.

Tab. 2. Mean food intake, body weight and epididymal fat pad weight in MS left vagus nerve stimulation (10Hz), sham operated, control (intact), and standard rats.

Second experiment	Sham	MS–10 Hz	Control	Standard
Food intake during experiment (g)	990±56	878±89*	1011±65	1210±89
Initial body weight (g) – day 1	467.3±25.1	470.1±26.7	478.7±23.8	526.5±25.2
Final body weight (g) – day 42	607.9±44.5	577.9±55.3*	612.7±49.5	629±22.7
Body weight gain (g)	140.6±37.8	111.8±36.1*	134.1±36.7	111.3±19.5
Body weight gain increment over initial body weight (%)	30.2	22.7*	28.9	20.8
Epididymal fat pad weight (g)	11.1±2.8	9.0±1.7*	10.5±2.7	9.3±2.6

Asterisks (*) indicate significant differences between MS–10Hz group and sham and control group.

Tab. 3. Blood serum levels of glucose (mmol/L), as well as aspartate (AspAT) and alanine (AlAT) transaminases (UI/L) in MS left vagus nerve stimulation (1 Hz and 10 Hz), sham operated, control (intact), and standard rats.

Group	1 Hz experiment			10 Hz experiment			
	Sham	MS–1 Hz	Control	Sham	MS–10 Hz	Control	Standard
Glucose	7.71±0.49	7.72±0.42	8.11±0.69	7.14±0.47	6.91±0.67	6.8±0.84	7.37±1.11
AspAT	153±19	186±17	160±28	185±32	185±42	195±40	170±53
AlAT	68±10	60±7	65±11	52±7	56±5	60±9	67±17

Blood biochemical analyses

There were no statistically significant differences between the VNS-treated rats and sham, control, as well as standard groups in the level of glycemia and in the levels of aspartate and alanine transaminases as well (Table 3).

Corticosterone

Corticosterone serum levels were increased in stimulated rats with both (1 Hz and 10 Hz) types of peripheral vagal stimulation. Vagus nerve stimulation with 10 Hz significantly elevated corticosterone serum levels compared to other groups of animals (222.96±63.52 ng/mL in stimulated group, 71.27±23.68 ng/mL in sham group and 46.58±26.66 ng/mL in control group, respectively; Figure 1). Vagus nerve stimulation (1 Hz) increased corticosterone serum levels, though not significantly, compared to sham 1 Hz and control 1 Hz groups (141.58±52.58 ng/mL in stimulated group, 73.18±27.87 ng/mL in sham group, and 48.31±10.64 ng/mL in control group, respectively; Figure 1). It appeared that the stimulation with higher frequency evoked stronger response of the hypothalamic-pituitary-adrenal axis. No differences were found between relevant sham and control groups as well as standard group (Figure 1).

Activated neurons in the nucleus of the solitary tract

c-Fos–positive neurons in the NTS were found in all of the rat brain hemisections examined. The amounts of c-Fos positive cells were similar in the NTS in the control, sham and standard groups. However, the number of c-Fos-positive cells increased significantly ($p<0.01$) above basal levels following left vagus nerve stimulation with 10 Hz frequency (Figure 2).

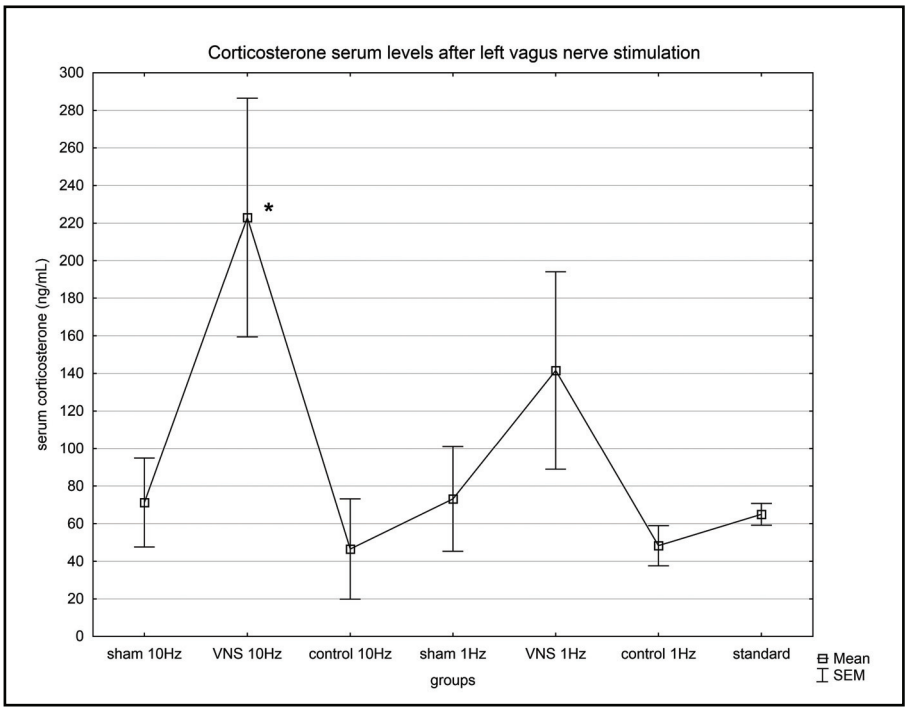

Fig. 1. Corticosterone serum concentration after left vagus nerve stimulation with the frequency of 1 Hz (MS-1Hz), 10 Hz (MS-10 Hz) and relevant sham, control and standard groups (n=8 for each group). Corticosterone levels significantly elevated in MS-10 Hz and in the MS-1 Hz groups. Data are presented as mean and standard error of mean (SEM). * $p<0.05$ as compared to relevant sham and control group.

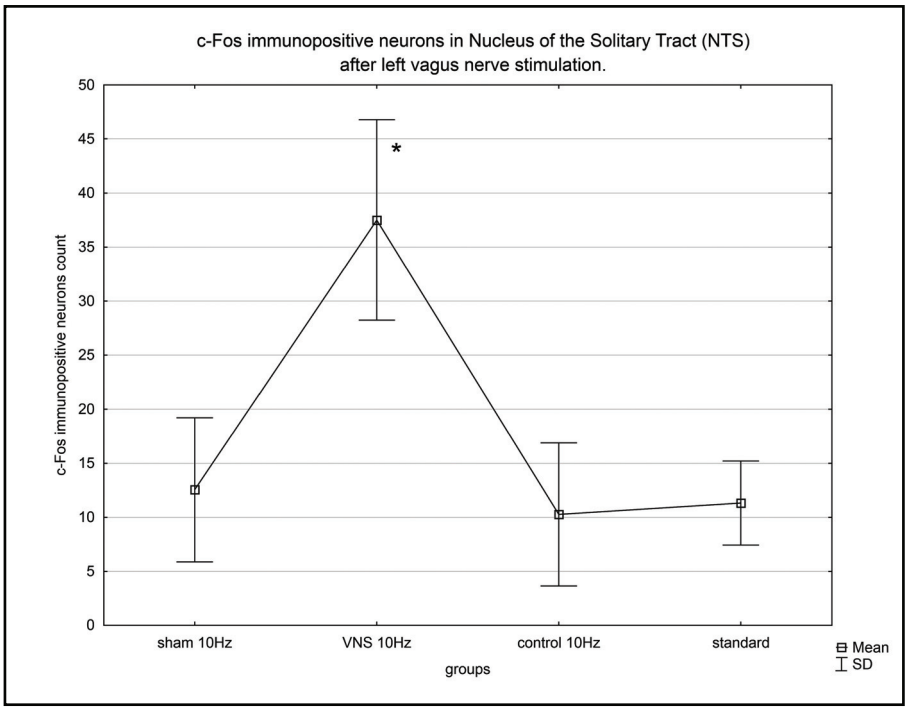

Fig. 2. The number of c-Fos immunopositive neurons in the nucleus of the solitary tract (NTS) after peripheral electrical stimulation of the left vagus nerve with the frequency of 10 Hz. The significant increase of c-Fos positive neurons in the NTS of stimulated rats. Data are presented as the mean and standard deviation (SD); n=8 for each group. Asterisks (*) indicate significant differences between the MS-10Hz group and the sham, control and standard groups, respectively.

DISCUSSION

In current study we evaluated effects of VNS on the basal activity of the hypothalamus-pituitary-adrenal-axis in rats fed high-fat diet. The influence of HFD on the basal HPA activity has been previously examined. Analysis by Auvinen *et al.* (2011) revealed, at least in mice models, conflicting results with respect to the hypothalamus-pituitary-adrenal-axis activation by high-fat diets. Plasma corticosterone levels were unchanged in 40%, elevated in 30%, and decreased in 20% of the studies examined. The effects in the peripheral tissues and the central nervous system were also inconsistent and major differences were found between mouse strains, experimental conditions, and the content and duration of the diets. This review demonstrated that the effects of high fat feeding on the basal activity of the hypothalamus-pituitary-adrenal-axis are

limited and inconclusive. Our experiment did not reveal significant changes in corticosterone levels caused by high-fat diet. Tannenbaum *et al.* (1997) reported an elevation of corticosterone concentrations after fatty diet in rats. In terms of HPA activity, increased fat consumption appeared to function as a chronic stressor. As in other chronic stress models, there appeared to be a small amount of adaptation to high-fat feeding. After 12 weeks of exposure to high fat, animals failed to show a significantly elevated basal corticosterone levels compared with controls.

Conversely, it has also been demonstrated that food deprivation markedly elevates the plasma level of corticosterone. Park *et al.* (2004) described elevated serum corticosterone concentrations in response to fasting in both fasted normal and spontaneous dwarf male rat. Chang *et al.* (2002) reported similar results in ovariectomized rats. Food restriction induced higher corticosterone plasma concentration through enhanced ACTH release. Additional data came from studies by Timofeeva *et al.* (2002), Jahng *et al.* (2005) and by Chmielowska *et al.* (2011). These data suggest that the responsivity of the HPA axis either to fasting or high-fat feeding stressors results in increasing corticosterone release.

Since VN participates in the HPA activation, and high-fat diet as well as food deprivation both increase corticosterone levels, it seems reasonable to assume, that when VN integrity is disturbed, the release of ACTH and corticosterone should be blocked. The experiments with vagotomy, however, brought confusing results. Kessler *et al.* (2012) examined serum levels of corticosterone in sepsis in mice. Vagotomy in the non-septic organism does not influence the corticosterone level. In the study by Bugajski *et al.* (2007) subdiaphragmatic vagotomy or sham vagotomy itself did not substantially alter the resting plasma ACTH and serum corticosterone levels, measured 2 weeks after surgery, but reduced ACTH and corticosterone secretion and brain norepinephrine changes that follow the intraperitoneal administration of LPS and IL-1β after subdiaphragmatic vagotomy in Wistar rats. This observation was in agreement with other reports by Hansen *et al.* (2000) after intraperitoneal lipopolysaccharide administration and by Arnhold *et al.* (2009) in water-restricted rats. These data indicate that an intact vagus is necessary to proper responses of HPA axis to stress factors. It should be also mentioned here, that in our experiments rats were kept in single cages, what could be considered as such stress factor and thus may influence the HPA axis tonicity (Bugajski *et al.* 2004). Such study design, however, allowed us to assess the amount of food consumed separately by each animal. Secondly, all animals were treated similarly, and thirdly, the VNS protocols lasted 6 weeks, and such long time supposed to be adequate to develop the appropriate adaptation.

In current study chronic vagus nerve stimulation significantly decreased food intake, body weight gain and epididymal fat pad weight in stimulated animals compared to control animals in both experiments with 1 Hz and 10 Hz VNS frequencies. Moreover, stronger effect of VNS with 10 Hz stimulation rate on such behavioral parameters has been noted. These data are consistent with previously reported results from experiments done in rats fed the standard diet (Bugajski *et al.* 2007), and further, following left VNS with 10Hz frequency in rats fed a high-fat diet (Gil *et al.* 2011a, b). Multiple animal models proved the efficacy of VNS in weight loss, whereas clinical studies are so far inconclusive (see review Bugajski & Gil 2012).

I has been reported, that VNS is involved in activation of the hypothalamic-pituitary-adrenal axis. This was demonstrated by VNS-induced increased hippocampal expression of corticotrophin releasing factor and increased plasma levels of adrenocorticotropic hormone and corticosterone after VNS by Hosoi *et al.* (2000) and de Herdt *et al.* (2009). In the study by Hosoi *et al.* (2000) direct electrical stimulation of the central end of the vagus nerve performed continuously for 2 h. induced increases in the expression of corticotrophin releasing factor mRNA in the hypothalamus. Furthermore, plasma levels of ACTH and corticosterone were also increased by this stimulation. This first report indicating that activation of the afferent vagus nerves itself can activate the HPA axis was consequently supported by work from O'Keane *et al.* (2005), performed in group of patients with chronic depression. Chronic depression increased ACTH response to CRH challenge. Short-term treatment with VNS therapy was associated with normalization of this response. Furthermore, De Herdt *et al.* (2009) investigated the effects of VNS on serum corticosterone levels following 1 h of high frequency (30 Hz) or low frequency (1 Hz) VNS in awake animals. He reported significant increase in serum corticosterone levels following 30 Hz VNS compared to 1 Hz VNS or sham stimulation. Recently Thrivikraman *et al.* (2012) in rats examined the left cervical VNS with current pulses set at 20 Hz. Acute VNS increased HPA axis activity, as well as c-Fos positive neurons count in NTS, with tendency toward recovery to baseline in animals treated by chronic VNS. These results suggest an immediate effect of VNS on the hypothalamic pituitary-adrenal (HPA) axis and support the role of the vagal nerve in immunomodulation as well as in maintaining organism's homeostasis. So far, there was lack of reports, either clinical or experimental, reviewing VNS related to HPA activity in connection to obesity. In current study we have found elevated basal corticosterone serum levels in stimulated rats with both (10 Hz and 1 Hz) types of peripheral vagal stimulation compared to sham and control groups. Noteworthy, it appeared that the stimulation with higher frequency evoked stronger response of the hypothalamic-pituitary-adrenal axis.

The VNS in the possibly new therapies has been extensively studied, including animal models. Although exact mechanisms of VNS central action

remain unclear, few possibilities have been discussed (Aalbers *et al.* 2011). All postulated mechanisms prove, that NTS neurons activation is the key factor in mechanism involving peripheral vagus nerve stimulation. The question arises, how to set – up the VNS parameters, to treat the patients in efficient, but concurrently safe way.

This issue was intensely discussed by Bonaz *et al.* (2013). It was firstly hypothesized that vagus nerve stimulation at high frequency (20–30 Hz) acts mainly on vagal afferents and thus is useful for the treatment of drug-resistant epilepsy and depression, while the low-frequency (less than 5 Hz) VNS targets predominantly vagal efferents and evokes anti-inflammatory effects (known as CAP – anti-inflammatory pathway). Moreover, the frequency significantly higher than 50 Hz was shown to be ineffective, possibly causing disruption of electrical properties vagal fibers and leading to its irreversible injury (Merrill *et al.* 2005).

Osharina *et al.* (2006) performed VNS of the central cut left VN. They showed a gradual increase in c-Fos expression in the brain of animals with VNS from 1 to 10 Hz; 1 Hz VNS only discretely affected the level of c-Fos expression in the NTS, compared to sham-operation, while in contrast, c-Fos expression was markedly above sham-operation levels in the NTS following 10 Hz stimulation. The first fMRI (functional magnetic resonance imaging) study of acute (1 h) VNS in anesthetized rodents revealed, that VNS at low-frequency stimulation (5 Hz), is also able to activate the brain neurons (Reyt *et al.* 2010). Thus, considering our results, we conclude, that left VNS with either lower (1 Hz) or higher (10 Hz) frequency is capable to influence the brain centers, but VNS with higher frequency seems to be more efficient in modulations of NTS neurons and HPA activation.

Finally, we wondered, whether peripheral VNS applied in our study was sufficient to influence brain circuits, imitating the satiety signals originating from the gut to the CNS, and thus, leading to decrease in food consumption and body mass reduction. Consequently, since the cell bodies of the vagal visceral afferents are contained within the nodose ganglions, and the central terminals of the primary vagal afferents are mainly found in the nucleus of the solitary tract, an increase in c-Fos positive neurons in the NTS may confirm our VNS to be efficient. Indeed, in the present study we revealed a significant response in the NTS following left vagus nerve stimulation. These findings along with previously published data (Gil *et al.* 2011b) on the presence of multiple c-Fos positive neurons in the nodose ganglions of stimulated rats indicate, that VNS evoked a response in the appetite-related neural centers.

The electrical peripheral stimulation of vagal afferents is the area of interest in the experimental and clinical obesity management. One of the possible mechanisms of VNS actions includes neuroendocrine modulation of the HPA axis.

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