INTRODUCTION

The body weight and body fat content are regulated by multiple factors (1-6). Body weight remains stable within relatively narrow range, despite large day-to-day fluctuations in the amount of food consumed. It is important to recognize, that short-term and long-term food intake and energy balance are regulated through different but interacting mechanisms. Short-term regulation signals have a different function than long-term that are activated in proportion to both adipose stored and energy consumed over a long period of time. In short-term regulation glucostatic hypothesis of regulation of food intake was proposed by Mayer over 50 years ago (7). Hypoglycemia or inhibition of glucose metabolism with 2-deoxy-D-glucose not only increases food intake but also stimulates vagal activity (8). Campfield and Smith first demonstrated that a vagally mediated spike of plasma insulin concentration induces small (10-15 mg/dl) transient decrease of blood glucose level that precedes spontaneous feeding in rats (9, 10). Eating can be induced by mimicking this effect by administering small amount of insulin. In addition, entry of food into the stomach and proximal small intestine activates stretch- and mechanoreceptors. These signals are transmitted also via vagal nerves to the hind brain, where they are integrated and play a major role in short-term regulation, by limiting the size of single meal (1, 2). These types of signal may also affect energy intake in subsequent meals.

We previously showed that short-term vagal stimulation affects volume regulation of food intake and decreases body weight in rats (11-13). Randich and Cox (14-16) using extracellular recordings from the vagus nerve showed, that vagus nerve conducts “satiety signal” from the jejunum, activated by fatty acid infusion. On the contrary, in humans subjected to VNS no changes in body weight were observed. There is also considerable evidence, that vagal afferent nerves can be activated by CCK, leptin, and ghrelin (17-24).

The purpose of current work was to evaluate the role of vagal nerve stimulation in long-term regulation of body weight and food intake in high fat diet induced obesity in rats. We examined also the effects of VNS on interstitial cells of Cajal (ICC), myenteric plexus neurons, mast cells in the stomach, duodenum and colon and c-Fos expression in nodose vagal ganglion. The effects observed during long-term VNS concern predominantly mast cells. These data support the theory that VNS can increase vagal afferent satiety signals leading to reduced food intake and body weight gain and mast cells are involved in this process.

Some previous studies have shown suppressive effect of the vagal nerve stimulation (VNS) on long-term feeding regulation in rats. We assessed body weight, interstitial cells of Cajal (ICC), myenteric plexus neurons, mast cells in the stomach, duodenum and colon and c-Fos expression in nodose vagal ganglia in the rats with VNS. Male Wistar rats were implanted with microchip (MC) and kept during the whole study (100 days) on high caloric diet. Left vagal nerve was stimulated by electrical pulses (10ms, 200mV, 0.05Hz) generated by MC. After finishing the experiments tissue samples (stomach, duodenum, colon and nodosal vagal ganglia) were taken. Mast cells were toluidine blue stained and counted in mucosa, muscularis externa and serosa. For immunostaining, antibodies for ICC (CD117), myenteric plexus neurons (PGP9.5) and c-Fos were used. Positive cells were assessed by image analysis. Chronic microchip vagal stimulation significantly decreased epididymal fat pad weight, meal size with effect on decreased weight gain in VNS rat. VNS significantly increased mast cells number in all examined parts of the gastrointestinal wall, mainly in the muscularis. There were no significant differences in ICC and myenteric plexus neurons between VNS and control. Expression of c-Fos in nodosal ganglia was higher in VNS group. The effects observed during long-term VNS concern predominantly mast cells. These data support the theory that VNS can increase vagal afferent satiety signals leading to reduced food intake and body weight gain and mast cells are involved in this process.

Key words: vagal nerve stimulation, food intake, body weight, fat pad, obesity, mast cells, interstitial cells of Cajal, myenteric plexus, nodose ganglion
housed in individual cages and all were fed the same 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. The rats were randomly divided into three groups: 1. rats with active microchip (MC) connected by electrodes with the left vagal nerve (MC group, n=6), 2. animals with inactive MC without electrodes on the vagal nerve (Sham, n=6), 3. intact rats without MC and electrodes (Control, n=6). To eliminate effects of surgical procedures and microchip implantation on morphological parameters (mast cells count, interstitial cells of Cajal, and myenteric plexus neurons) control group of unoperated animals (intact group) was included in the study. Sham operated group served as the most important reference group for the assessment of the food intake, body weight and epididymal fat pad weight. After about 3 weeks (day 25th) of operation the assessment of the food intake, body weight and epididymal fat pad weight was carried out.

**RESULTS**

**Food intake and body weight**

Electrical stimulation of the left vagal nerve reduced the daily and total food intake in MC group compared with sham group, but the differences were not statistically significant (p=0.37). No differences between MC and control group were observed. (Table 1).

Consequently to reduced food intake, final body weight was lower, but not significantly, in group with VNS (MC) compared with no stimulated group (sham) (p=0.30) but not with control animals (Table 1).

**Epididymal fat pad weight**

Fat pad weight reflecting the total body fat content was significantly higher in sham group compared to MC group (p<0.05). For control group fat pad weight was slightly higher than in MC group, but these data were not significant. Mean epididymal fat pad weight relative to body weight (fat pad/body weight ratio) was significantly lower (27.4%) for rats with active MC as compared to sham animals (p<0.01), but not to control group (Table 1).

**Mast cells**

In the stomach wall VNS significantly increased total mast cells number compared to sham and control groups (282.2 ± 168

### Table 1. Food intake, body weight and epididymal fat pad weight in microchip left vagus nerve stimulation (MC), sham operated and control (intact) rats

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MC</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td>Food intake during exp. (g)</td>
<td>1997 ± 203</td>
<td>1896 ± 171</td>
<td>1876 ± 215</td>
</tr>
<tr>
<td>Initial body weight – day 1 (g)</td>
<td>373.3 ± 14.1</td>
<td>376.5 ± 12.9</td>
<td>370.1 ± 21.4</td>
</tr>
<tr>
<td>Final body weight - day 102 (g)</td>
<td>688.7 ± 76</td>
<td>651.7 ± 63</td>
<td>669.9 ± 57</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>315.8 ± 27.4</td>
<td>291.2 ± 29.2</td>
<td>290.8 ± 19.7</td>
</tr>
<tr>
<td>Epididymal fat pad weight-EFP (g)</td>
<td>13.8 ± 3.5</td>
<td>10.2 ± 2.5</td>
<td>11.0 ± 2.6</td>
</tr>
<tr>
<td>EFP/body weight ratio</td>
<td>0.020</td>
<td>0.0157</td>
<td>0.0166</td>
</tr>
<tr>
<td>EFP/body weight ratio increment over MC rats (%)</td>
<td>127.4</td>
<td>100</td>
<td>105.7</td>
</tr>
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</table>
in MC group, 192.3 ± 103.7 in sham group and 188.7 ± 57.2 in control). The highest values were observed in muscularis externa (180.8 ± 98.9 in MC group, 131.5 ± 67.8 in sham group and 124.3 ± 41.1 in control) (Fig. 1).

In the duodenum mast cells number after VNS (16.0 ± 9.05) remained unchanged compared to sham (18.0 ± 8.6) and control (23.7 ± 22.8) as well as in proximal colon (MC – 18.7 ± 13.4; sham – 17.3 ± 11.6; control – 22.3 ± 22.1). No significant differences between VNS group and control groups were observed.

**Interstitial cells of Cajal**

The area of the ICC in myenteric plexus region stained with CD117 differs in each region of gastrointestinal tract of the rats (for control group: stomach – 6688 ± 2467 µm²; duodenum – 6534 ± 3545 µm²; colon – 10590 ± 4464 µm², for sham group: stomach – 7008 ± 2645 µm²; duodenum – 7212 ± 3639 µm²; colon – 8801 ± 3406 µm², and for MC group: stomach - 7428 ± 2804 µm²; duodenum – 7690 ± 3983 µm²; colon – 10742 ± 3790 µm²). No significant differences between VNS group and control groups in each part GI tract were observed (Fig. 2).

**Myenteric plexus**

The area of the myenteric plexus stained with PGP 9.5 was different in each region of gastrointestinal tract of the rats (for control group: stomach – 11215 ± 5210 µm²; duodenum – 6745 ± 2185 µm²; colon – 9744 ± 4910 µm², for sham group: stomach – 10809 ± 5437 µm²; duodenum – 5740 ± 3043 µm²; colon – 7456 ± 3533 µm², and for MC group: stomach – 9039 ± 5669 µm²; duodenum – 6102 ± 3412 µm²; colon – 9351 ± 6180 µm²). However, no statistical differences between VNS group and control groups in each part GI tract were observed (Fig. 3).

**c-Fos positive cells in nodose ganglion**

Assessment of c-Fos positive neurons in nodose ganglia of vagus nerve showed significant increase in percentage of

**DISCUSSION**

Our experimental study was performed using high fat diet because obesity induced by high-fat diet mimics obesity in humans (25). Effect of short-term vagal neuromodulation in standard diet fed rats was studied previously (11-13). Vagal nerve stimulation by MC leads to a decrease in food intake in rats combined with a decrease in body mass when compared to sham operated animals with inactive microchip implanted, but
not intact group. These effects may be explained by "imitation" of the physiological input associated with gastric mechanoreceptors and jejunal chemoreceptors activation by food. VNS may mimic the physiological input associated with gastric mechanoreceptors and vagal nerve and the mast evidence, that mast cells and endings of vagal fibers are co-
role of vagus nerve–mast cells interaction in the stomach was
demonstrated by Stead in a series of experiments showing that vagal afferents penetrate the small intestinal mucosa and contact interstitial mucosal mast cells, moreover, vagotomy causes a reduction in mast cells density, suggesting a trophic effect, and stimulation of the cervical vagus causes an increase in histamine and serotonin in mast cells (45).
That is consistent with our data showing an increase in mast cell number after VNS and demonstrates an important role of mast cells in the transmission of the satiety signals by afferent vagal fibers, which needs to be elucidated. Further understanding the mast cell–vagus axis may be important in the development of treatments for various human diseases, including functional bowel disorders.

In the present study we examined the interstitial cells of Cajal localized in myenteric plexus region in the stomach, duodenum and proximal colon and the myenteric plexus neurons as well. We did not find quantitative differences caused by VNS. Previously, we have shown that chronic vagal stimulation significantly decreased ICC number in the proximal colon in rats (46). Current data did not confirm those results. We assume that damage of ICC in proximal colon was caused by higher voltage (0.55 V) and longer duration of impulse (100 ms) generated by previously used microchip.

The present study demonstrates that food intake and body weight gain may be decreased by long-term vagus nerve stimulation in the high-fat diet induced obesity in rats. These data support our hypothesis that artificial electrical signals generated by MC and conducted by vagal afferents as satiety signals, can modify central regulation of food intake and body fat content. Peripheral mechanisms, concerning mast cells are also involved in this process. Vagal neuromodulation seems to be a promising method for obesity treatment (47-49).

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REFERENCES


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