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A.J. BUGAJSKI, K. GIL, A. ZIOMBER, D. ŻUROWSKI, W. ZARASKA[1], P.J. THOR

EFFECT OF LONG-TERM VAGAL STIMULATION ON FOOD INTAKE AND BODY WEIGHT DURING DIET INDUCED OBESITY IN RATS

Department of Pathophysiology, Jagiellonian University Medical College, Cracow, Poland,
[1]Institute of Electron Technology, Cracow, Poland

Regulation of food intake and body weight is accomplished by several mechanisms. CNS receives information from periphery and modifies food intake mainly by vagal nerves that provide the major neuroanatomical link between gastrointestinal sites stimulated during food intake and CNS sites that control feeding behavior and metabolism. Gastric mechanoreceptors and jejunal chemoreceptors activated by food or vagal nerve stimulation (VNS), which mimic the physiological input, suppress feeding within short-term regulation. Our research was aimed on determination the role of electrical VNS in long-term control of food intake and body weight in diet induced obesity fed rats. Food intake, body weight and epididymal fat pad were assessed in male Wistar rats divided into three groups (controls vs. VNS). Rats were implanted with microchip and kept during the whole study (100 days) on diet induced obesity. Vagal nerve was stimulated by electrical rectangular pulses duration 10 ms, amplitude 200 mV, frequency 0.05 Hz generated by microchip. In control group surgery produced no significant changes in meal size and body weight gain as compared to intact group. In contrast, significantly decreased epididymal fat pad weight, decreased meal size with effect on decreased weight gain was observed in VNS rats. Data support theory that VNS can increase vagal afferent signal conduct to CNS and mimics the satiety signals leading to reduce food intake and body weight gain.

Key words: *vagal nerve stimulation, microchip, food intake, body weight, fat pad, obesity*

INTRODUCTION

There is considerable evidence that body weight and body fat content are under mutifactorial regulation (1 - 6). Body weight remains 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 deoksy-D-glucose not only increases food intake but also stimulates vagal activity (8). Campfield and Smith first demonstrated that small (10-15 mg/dl) transient decrease of blood glucose preceded spontaneous feeding in rats, which is preceded by vagally mediated a spike of plasma insulin concentration (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 activate stretch- and mechano-receptors. These signals transmitted also via vagal nerves to the hind brain where they are integrated and have 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 a 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 recording from the vagus nerve, clearly shown, that vagus nerve conduct “satiety signal” from the jejunum, activated by fatty acid infusion. Contrary to this observation, in humans subjected to VNS no changes in body weight were observed. It is well known that vagal afferent nerves can be activated by CCK, leptin, ghrelin (17 - 24).

This study was aimed to evaluate the role of vagal nerve stimulation (VNS) in long-term regulation of body weight and food intake in high fat diet induced obesity in rats.

MATERIAL AND METHODS

Eighteen Wistar male rats with mean body weight at the beginning of the study 374 ± 18 g were used. The animals were housed in individual cages and were fed with diet induced obesity (DIO) (Perform, Bento Kronen Products, Belgium) with higher percentage of fat than in the standard diet. 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 a temperature 23 ± 2°C on a 12:12-hour dark/light cycle. Jagiellonian University Bioethical Committee approved care and use of the animals.

The rats were randomly divided into three groups used in experiment: 1. the group with active microchip (MC) connected by electrodes with the left vagal nerve (n=6), 2. the group with inactive MC without electrodes on the vagal nerve (n=6), 3. the intact group -without MC and electrodes (n=6). After about 3 weeks rats with the average mass 478 ± 46 g were operated after 12 hours of deprivation of food in the general anesthesia with pentobarbital given intraperitoneally (Vetbutal, 0.25 mg/kg, Biowet, Puławy, Poland). The MC for chronic vagal stimulation (Institute of Electron Technology, Cracow) was placed into the subcutaneous pocket and the ends of the MC platinum

electrodes were wrapped around the subdiaphragmatic left vagal nerve; cathode and anode were positioned at 0.5 cm distance. In the control group inactive MC was implanted and laparotomy was performed. The third group of rats was no operated (intact group).

The impulses parameters generated by MC were: unipolar rectangular pulses duration 10 ms, amplitude 200 mV, frequency 0.05 Hz. The parameters of MC stimulation were based on our previous experiments (11, 13). Daily food intake and body weight were measured each morning. The amount of daily food intake was determined by subtracting the amount of food remaining from that given 24 h before. Food was withdrawn on the day before surgery and was restored on the day after operation.

At the end of the experiment all the rats were killed by decapitation and weighed. Additionally both epididymal fat pads, located between the cauda epididymis and the distal extremity of the testis, were dissected from the animal and weighed. The proportional weight of the fat pads was calculated by dividing the fat pad weight by the total body weight.

Data are shown as mean ± SE. To determine the significance between different groups, 1-way ANOVA or t-student test were performed. A value of P 0.05 was considered statistically significant.

RESULTS

Food intake

All rats decreased their food intake during the first two days after surgery. This decrease in average food consumption (21-st and 22-nd days of experiment) was associated with a 12-hours food deprivation before MC implantation and with surgery. Electrical stimulation of the left vagal nerve reduced the total food intake (*Table 1, 2*) and also daily food intake, but they were not statistically significant.

Body weight

All rats lost up to 10 % of their weight during 5 days after surgery and subsequently followed weight gain curve. Consequently to reduced food intake, final body weight was lower in group with VNS compared with no stimulated controls (*Fig. 1, Table 1, 2*).

Table 1. Food intake and body weight of rats with active microchip during post-surgery period (day 25-103). Final body weight is the weight of rats on the day of sacrifice.

Rat number	Food intake (g)	Initial body weight day 25 (g)	Final body weight day 103 (g)	Body weight gain after surgery (g)
8	1814.5	463.5	610.5	147
9	2214.3	483.5	752.1	268.6
11	1913.5	505.8	658.6	152.8
12	1900.4	440.5	611.7	171.2
13	1823.5	460.5	693.1	232.6
15	1713.1	448.5	584.2	135.7
mean	1896.5 ± 171	467.05 ± 24	651.7 ± 63	184.6 ± 54

Table 2. Food intake and body weight of rats with inactive microchip during post-surgery period (day 25-103). Final body weight is the weight of rats on the day of sacrifice.

Rat number	Food intake (g)	Initial body weight day 25 (g)	Final body weight day 103 (g)	Body weight gain after surgery (g)
1	2042.5	472.5	667.2	194.7
2	1718.9	468.6	603.8	132.5
3	1766.5	452.2	610.2	158
4	2176.3	525.5	801.5	276
6	2112.9	470.4	716.9	246.5
14	2164.9	514.3	732.7	218.4
mean	1997 ± 203	483.9 ± 29	688.7 ± 76	204.3 ± 53

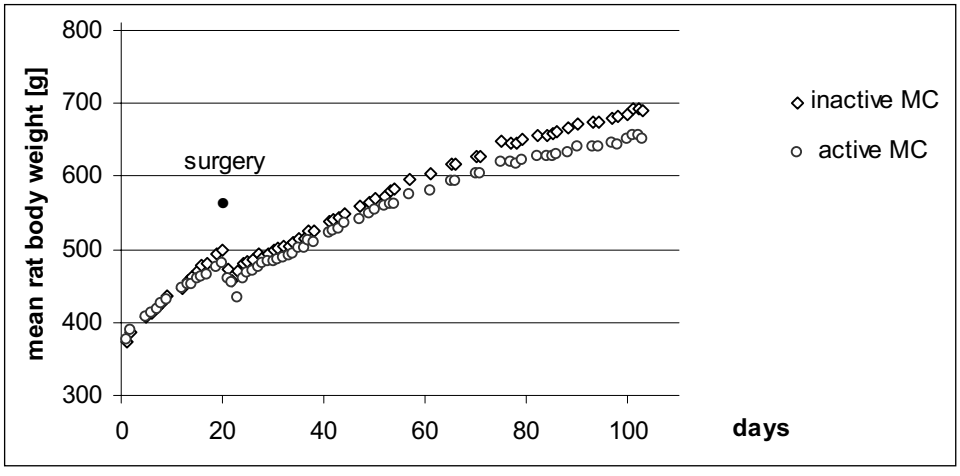

Fig. 1. Mean daily body weight before surgery (day 1-20) and after surgery (day 21-103). Average body weight was calculated for 6 rats in each group. Intact group is not shown, but this group values were almost equal than in group with inactive microchip (MC).

Epididymal fat pad weight

Fat pad weight reflecting the total body fat content (*Table 3*). Fat pad/body weight ratio was significantly lower in rats with active MC groups.

Mean epididymal fat pad weight relative to body weight (fat pad/body weight ratio) was 23.1% lower for rats with active MC as compared to controls (*Fig. 2*).

DISCUSSION

We decided to perform study using high fat diet because obesity induced by high-fat diet mimics obesity in humans (25). Effect of short-term vagal

Table 3. Body and epididymal fat pad weight on the day of sacrifice. Values are expressed as mean ± SE. n=6 each group. MC= microchip

Variables	Inactive MC	Active MC	Intact
Baseline, g	373.3 ± 14.1	376.5 ± 12.9	370.1 ± 21.4
Day 100, g	688.7 ± 76.5	668.4 ± 53.9	609.9 ± 56.7
BW gain, g	315.8 ± 27.4	291.2 ± 29.2	239.8 ± 19.7
Epididymal fat weight, g	13.8 ± 3.5	10.2 ± 2.5	10.5 ± 2.6

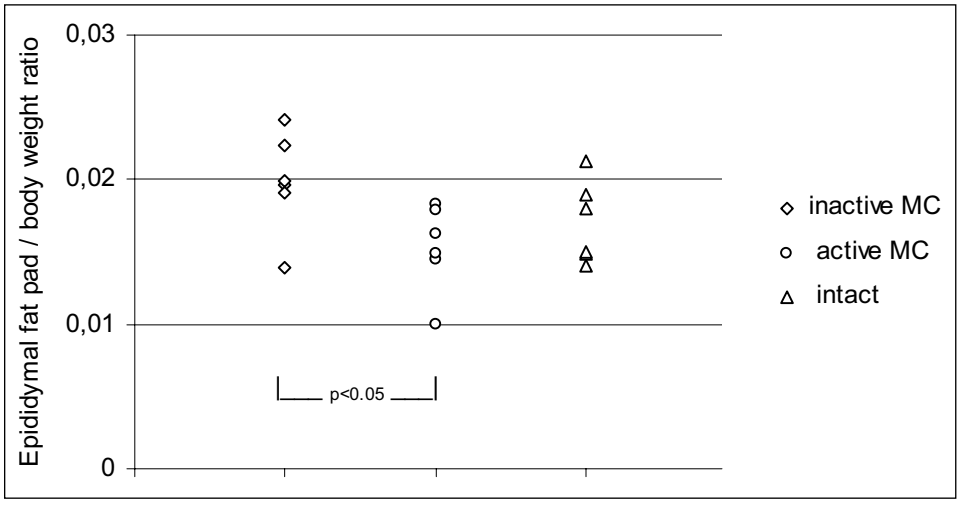

Fig. 2. Epididymal fat pad/body weight ratio in rats with inactive microchip (MC), active MC and in intact rats. n=6 for each group. Fat pad/body weight ratio reflects total body fat content. Epididymal fat pad/body weight ratio was calculated by dividing the fat pad weight by the total body weight.

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. These effects may be explained by „imitation" of the physiological input associated with gastric mechanoreceptors activation by food. VNS may mimic the physiological input associated with gastric mechanoreceptors and jejunal chemoreceptors (3, 26) activation by food and lead to decrease in food intake and subsequently decrease in weight gain.

Vagus nerve has unique abilities, consists mainly of afferent fibers which conduct signals from gut mechano- and chemoreceptors (26, 27). On the other hand on the vagal afferents are located receptors for cytokines, CCK (17 - 19), ghrelin (23, 24) and numerous of peptides released by meal.

However, some of the authors are convinced that vagus transfer hunger signals that promote feeding (28). Results of vagal stimulation obtained in humans are

also contradictory. In treatment of depression or epilepsy VNS did not affect body weight (29 - 31). Opposite results published Burneo, which observed weight loss in patients with epilepsy treatment by VNS (32). This discrepancy may be caused by different parameters of vagal stimulation or by side effects of VNS (32).

Peripheral effects of VNS cannot be excluded such as release of pancreatic polypeptide and insulin. Release of these hormones depends probably upon the frequency of stimulation. These signals transmitted also *via* vagal nerves to the hind brain where they are integrated and have major role in short-term regulation by limiting size of signal meal (33). Therefore, hypothesis could be made that vagal nerves are involved in both short-term and long-term food intake control. These types of signal may also affect energy intake in a subsequent meals. However, when the energy density of food was decreased over long period of time by more frequent but smaller meals consumed, such energy intake remains constant (34). Thus volume detection does not appear to have a major role in the long-term regulation of energy homeostasis. We previously showed that low frequency vagal stimulation affects short-term volume regulation of food intake and decreases body weight in rats (11 - 13).

In our data we observed decrease in body weight and food intake in long-term VNS rats.

Weight changes are characterized by alternations in adipose tissue mass. In rodents adipose tissue is localized in different depots. A particular depot of visceral fat is localized in the epididymal fat pad, which is well delimitated and easy to excise.

Epididymal fat pads represent only a small part of total body weight but previous studies have shown that the epididymal fat pad weight as a proportion of total body weight is highly correlated with total body fat in mice and rats. (35 - 37). The additional reason for including the caudal epididymal fat pad weight as one of the parameters was that fat pads reflects total body fat mass and are under hormonal and nervous regulation (22, 38 - 41). However, body composition was altered by vagal nerve stimulation (VNS) as expected: epididymal fat pad weight relative to body weight was significantly lower for rats with VNS compared with control rats. Peripheral neuronal reporters of satiety, originating with mechanical and chemical signals from the gut and liver, are primarily conveyed *via* vagal afferents to the nucleus of the solitary tract resulting in the activation of both ascending and descending circuits involved in food intake.

The present study demonstrates that food intake and body weight gain are decreased by long-term vagus nerve stimulation in the high-fat diet induced obesity in rats. These data support our main hypothesis that CNS can be deceived by artificial electrical signals generated by MC and conducted by vagal afferents as a satiety signals, suggesting mainly central regulation involved in food intake. Peripheral mechanism like visceral vagal afferents cannot be ignored and vagal neuromodulation is a promising method for future treatment of obesity in humans (42).

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Author's address: Andrzej Bugajski, M.D. Ph.D. Department of Pathophysiology, Jagiellonian University Medical College, Czysta 18 Str., 31-121 Cracow, Poland.
E-mail: mpbugajs@cyf-kr.edu.pl