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THE EFFECTS OF BACLOFEN ON THE FEEDING BEHAVIOUR AND BODY WEIGHT OF VAGALLY STIMULATED RATS

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It is hypothesised that the GABA(B) receptor agonist baclofen increases or has no effect on food intake, and electrical stimulation of vagal nerves decreases food intake. The aim of this study was to evaluate the effects of baclofen in vagally stimulated rats. Material and methods: Thirty two Wistar rats were divided into five groups: group A scheduled for microchip implantation for vagal stimulation, group B for sham operation, group C for microchip implantation and baclofen medication, group D for baclofen medication only and group E for gastric motility evaluation under influence of baclofen. The following parameters were then evaluated: food intake and body mass, gastric motility, leptin, insulin, and glucose serum levels. Results: In the comparison of groups B and A, daily food intake and body weight gain decreased by 17% ($p < 0.05$) and by 22% ($p < 0.05$), respectively. Baclofen alone (group D) did not significantly change either food intake nor diurnal body weight compared to the controls, but when used in conjunction with the microchip (group C) it did significantly reduce effect of vagal neuromodulation ($p < 0.05$). Furthermore, a significant decrease in leptin and glucose levels was detected in group C: 677 to 165 pg/ml ($p < 0.05$) and 5,93 to 4,88 mmol/l ($p < 0.05$), respectively. The administration of baclofen stimulated significantly gastric motility and elicited irregular motor migrating complex (327 ± 200 against control 255 ± 52 cmH₂O/s). Conclusions: These results suggest that microchip vagal neuromodulation through increased vagal afferent activity induces an alteration in the feeding behaviour and decreases nocturnal food intake and body weight. These effects were partially attenuated by baclofen. The data suggests that GABA(B) receptors play an important role in the pathomechanism of attenuation of food intake induced by vagal nerve stimulation.

Key words: *food intake, vagal stimulation, vagal physiology, feeding behaviour, baclofen.*

Abbreviations: microchip stimulator (MC), cholecystokinin (CCK), nucleus tractus solitarius (NTS), gamma amino butyric acid (GABA), gastrointestinal (GI)

INTRODUCTION

Mechanisms of food intake control are multifactorial and complex. Interactions between the peripheral and central mechanisms are of particular importance. The suprachiasmatic nucleus receives input *via* the retino-hypothalamic tract and synchronizes the intrinsic diurnal oscillator to light or darkness. It acts as an internal clock and has profound effects on the ingestive behaviour through various brain structures in a species-specific fashion (1,2). The majority of neurons in this area are GABA-ergic and activation of GABA receptors can induce shifts of the circadian oscillator (3).

Peripheral signals are mostly related to sensory inputs from mechano- and chemoreceptors of the gastrointestinal (GI) tract (4). In the short-term regulation of food intake, the stomach and small intestine act as nutrient reservoirs and initiate satiation by the augmentation of peripheral signals and determinate meal termination. Dominant sensory input involved in this transmission, known as the gut-brain axis, is conducted mainly by vagal nerves. Most of the gastric derived vagal afferents terminate in nodose ganglia, rich in GABA(B) receptors. They directly inhibit input from gastric mechanoreceptors and modulate feeding behaviour (3-6). The size and chemical content of the food induces a negative feedback loop which thus determines termination of the meal (7,8).

Electrical modulation of the vagal afferent discharge decreases food intake in experimental animals and humans (9-13). It is hypothesised that the GABA(B) -receptor agonists induces change in hypothalamic neurotransmission which leads to a decrease in food intake (inhibition of vagal mechanoreception) and affects the circadian rhythm of body weight gain in rats (3). The aim of this study was to evaluate the effects of baclofen in vagally stimulated rats.

MATERIAL AND METHODS

Animal subjects and evaluation of food intake.

Thirty two male Wistar rats were included into the study. Body mass of the animals ranged from 186 to 222 g. Animals were housed individually in transparent cages at a constant temperature of 23°C. Light-dark cycle was provided a 12:12 hours. Animals had free access to standard laboratory food (Labofeed, Kcynia, Poland) and water. Body mass and food intake was measured twice a day at 8.00 AM and 8.00 PM. Baseline values of food intake and body mass were taken (for three days) after two weeks of adaptation to laboratory surroundings, food and staff.

Surgical procedures.

Animals were divided into five groups: group A scheduled for microchip implantation for vagal stimulation, group B for sham operation, group C for microchip implantation and baclofen medication, group D for baclofen medication only and group E for gastric motility evaluation under influence of baclofen. Animals were anesthetized with pentobarbital 0.25mg/kg (Vetbutal, Biowet, Pulawy, Poland). Autonomic MC (IKE-OBR, Krakow, Poland) with battery (diameter of 11mm and

weight of 2,8g) was implanted subcutaneously in the dorsal area of the body. Two pairs of monopolar electrodes connected to the MC were positioned on both abdominal vagal nerve trunks (subdiaphragmatically) at a distance of 5 mm. The following current parameters were used: frequency 0.05 Hz, impulse duration 0.1s, and amplitude 0.55V. Parameters of the current used were chosen according to earlier experiments (10). Effects of MC stimulation on the GI tract had been evaluated in previous studies (9) but not in this study in order to minimise stress to the animals and avoid possible bias.

Baclofen (Polpharma S.A., Warsaw, Poland) was injected intraperitoneally, in doses of 2 mg/kg/day every morning (minimal dose with proved central effect (14)) throughout a period of 3 weeks

Gastric pressure recordings

The gastric motility was measured by mean of balloon (1cm³) introduced to the stomach *via* previously implanted stainless-steel fistula in group E before and after administration of baclofen and expressed as motility index [cmH₂O*s/ min]. Gastric contractions were recorded and cleared by PowerLab/8SP system and software.

Blood sample testing.

Blood samples were taken after overnight fasting. Serum leptin levels were measured by enzyme-linked immunosorbent assay (ELISA - Quantikine M, R&D System Inc.) and expressed in pg/mL. Insulin levels expressed in μU/mL were measured by radioimmunoassay. Glucose levels expressed in mmol/L were measured by oxidation-reduction method. All tests were done at the end of the experiment.

Statistical analysis.

The results are expressed as mean and standard deviation (±SD). The differences between the tested groups were analysed by a two-way repeated measure analysis of variance, followed by a Tukey post hoc test. Unpaired t tests were used to compare tested group and controls. Statistical significance was set at p<0.05 for all tests.

The study has been approved by the Animals Ethical Committee of Jagiellonian University (Krakow, Poland).

RESULTS

In comparison between group B and group A, daily food intake and body weight gain decreased by 17% (p<0.05) and by 22% (p<0.05), respectively (*Fig. 1,2*). The change was more significant at night time (*Table 1,2*).

Baclofen alone (group D) compared to controls did not significantly change food intake and diurnal body weight, but it significantly reduced the effect of MC (comparison between group C and D for both food intake and body weight gain at night, p<0.05). Changes of mean body mass in groups during period of the study were shown in *Table 3*.

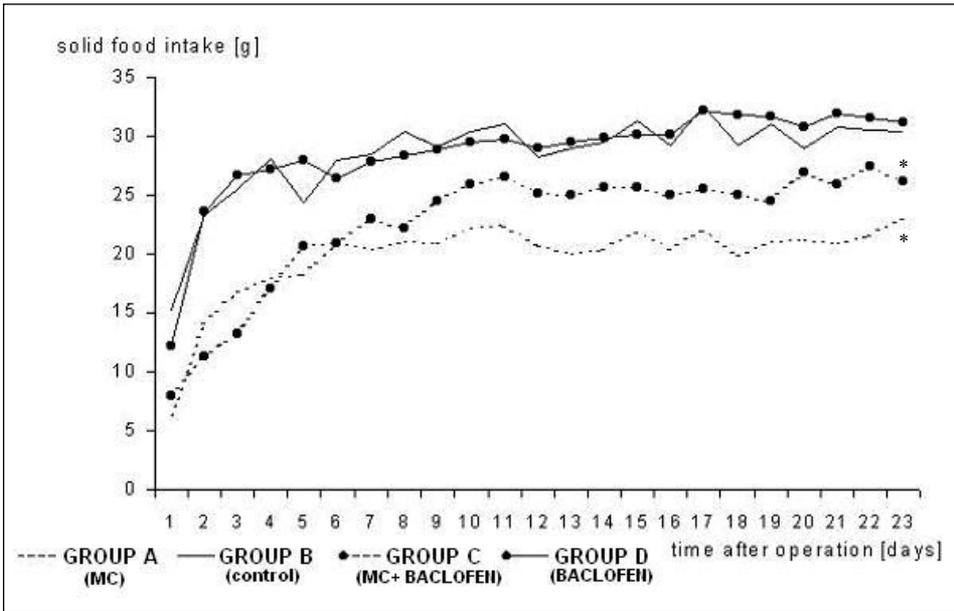


Fig.1. Solid food intake changes in groups A,B,C,D following 24 days after operation in four studied groups.

*- statistically significant ($p < 0.05$)

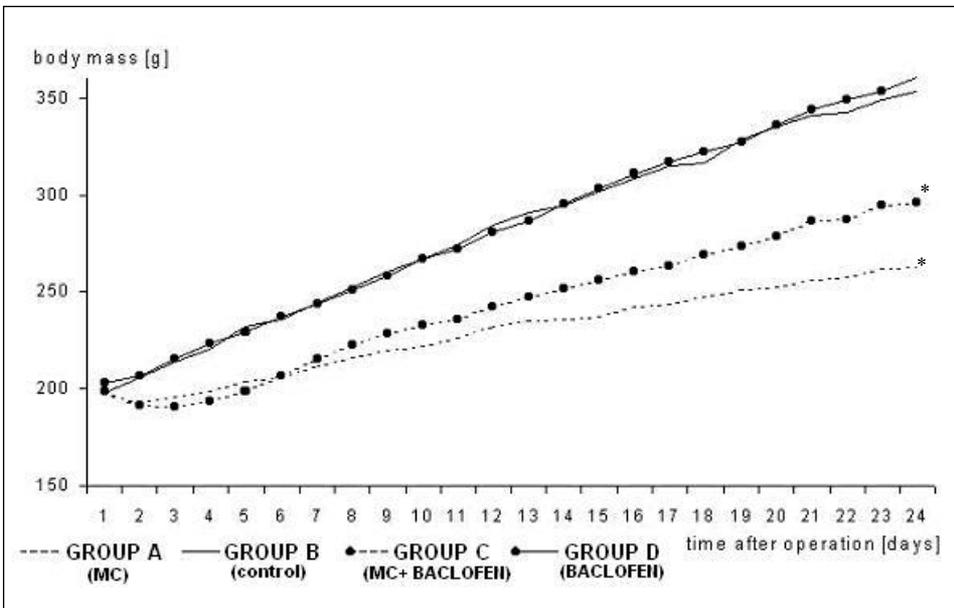


Fig.2. Body mass changes in the following 24 days after operation in groups A, B, C, D.

*- statistically significant ($p < 0.05$)

Table 1. Diurnal mean daily food intake [g].

GROUP	A-MC	B-Control	C-MC+Baclofen	D-Baclofen
On light	4.2±1.2	4.9±3.2	4.7±1.4	4.2±1.2
On dark	15.6±2.1*	24.7±2.6	18.1±1.8*	25.8±1.9
Total daily	19.8±1.9*	29.6±3.8	22.8±2.9*	29.9±1.6

*- statistically significant (p<0.05)

Table 2. Diurnal mean body weight gain [g] in all tested groups on light, dark and total daily gain.

GROUP	A-MC	B-Control	C-MC+Baclofen	D-Baclofen
Loss on light	-6.5±1.3	-10.8±2.7	-2.9±3.3*	-8.3±2.7
Gain on dark	9.3±1.4*	17.4±2.2	7.1±2.6*	14.8±2.2
Total daily	2.8±1.6*	6.6±2.7	4.2±2.2*	6.5±1.3

*- statistically significant (p<0.05)

Table 3. Mean body weight in the first (0) and the last (24th) day of experiments.

GROUP	0 day	last day(24th)	% of change
A-MC	196.8±15.0	262.7±36.3*	133.4
B-Control	197.7±16.1	353.5±22.4	178.8
C-MC+Baclofen	198.6±12.8	296.6±21.1*	149.3
D-Baclofen	202.7±8.1	361.1±39.5	178.1

*- statistically significant (p<0.05)

The administration of baclofen stimulated significantly gastric motility and elicited irregular motor migrating complex (327±200 against control 255±52 cmH₂O*s/ minute, p<0.05) (Fig. 3,4,5).

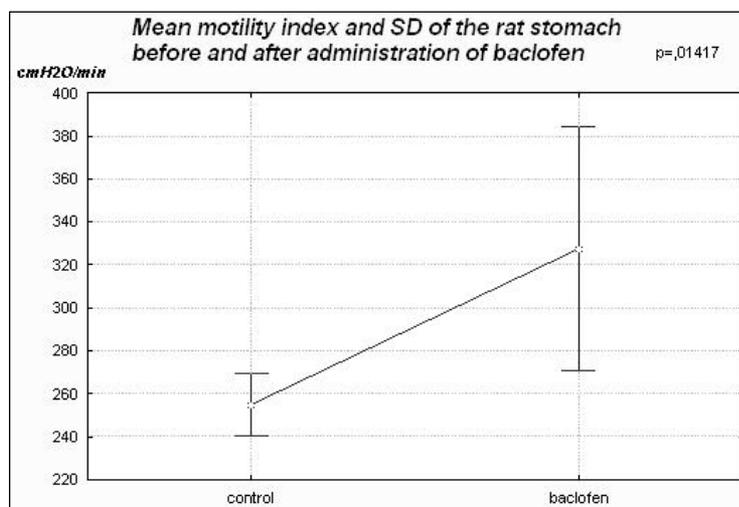


Fig.3. The gastric motility measured by mean of motility index (MI) in control group and after baclofen treatment. MI expressed as relationship between intragastric pressure measured in cmH₂O and time calculated for 1 minute period.

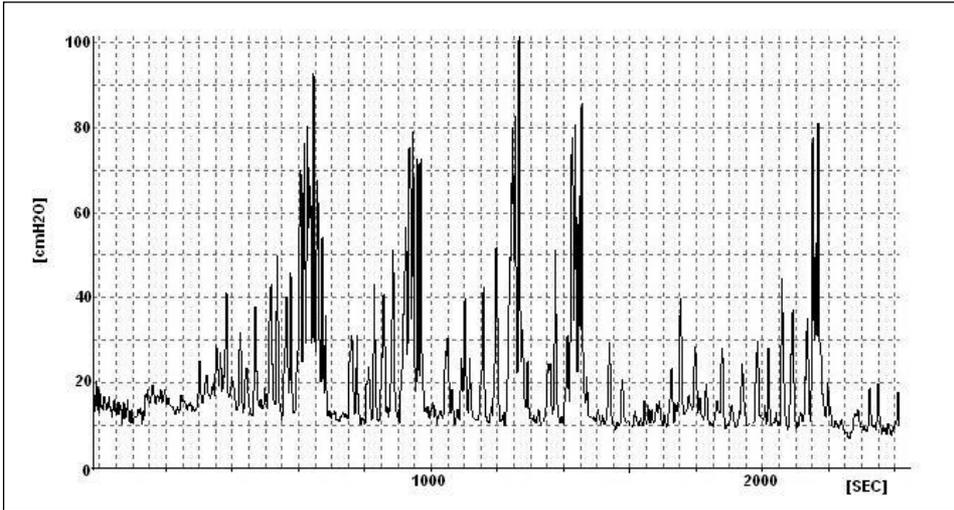


Fig.4. The gastric pressure changes in group E (rats with gastric fistula) after pre-treatment with baclofen in dose of 2mg/kg/day i.p. throughout a period of 3 weeks. Augmented motor migrating complex is clearly shown.

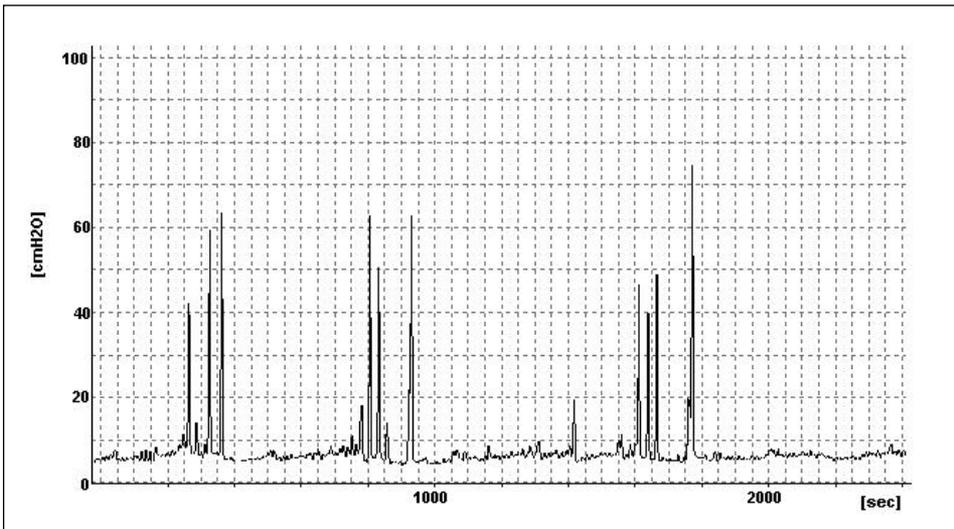


Fig.5. The gastric pressure changes in fasted control group of rats (group B). It showed normal propagation of motor migrating complex.

Significant decreases of leptin and glucose levels compared to controls were detected in group C (MC implants treated with baclofen) 677 to 165 pg/ml ($p < 0.05$) and 5,93 to 4,88 mmol/l ($p < 0.05$) respectively (Table 4). Insulin levels followed changes in glucose serum concentrations (Table 4).

Table 4. Mean leptin, insulin and glucose levels in each tested group at the last day (24th) of the study.

GROUP	Leptin concentration [pg/ml]	Insulin concentration [uU/ml]	Glucose concentration [mmol/l]
A-MC	625±254.7	4.32±0.7	5.27±1.24
B-Control	677±142.4	4.55±0.3	5.93±0.86
C-MC+Baclofen	165±128.0*	5.0±0.5	4.88±0.22*
D-Baclofen	612±174.3	4.52±0.3	6.03±1.37

*- statistically significant ($p < 0.05$)

DISCUSSION

Vagal afferents are the predominant neural pathway which carries satiation signals from GI tract. Vagal mediated satiation is augmented by cholecystokinin (CCK) by both central and peripheral mechanisms (16,17). There was also proven presence of leptin receptors in the nodal ganglion in rats (18). Interaction between leptin and CCK leads to suppression by CCK-1 receptors in capsaicin sensitive vagal fibers (19). It is suggested that vagal leptin receptors could be activated by adipocyte-derived leptin and by leptin produced in the stomach (20). Our data shows that MC vagal stimulation affected both food intake and body weight (mainly nocturnal). The fact that MC stimulation alone affected predominantly night over daytime feeding suggests a different response in the central circadian mechanisms of non-stimulated or chronic stimulated animals. Energy homeostasis is maintained by co-ordination mechanisms that regulate food intake through the changes in autonomic and endocrine functions and is focused on sustaining an appropriate state of arousal (15). The hypothalamus plays a critical role in maintaining energy homeostasis by integrating sensory input of metabolic, neuroendocrine and behavioral responses. Mintz *et al.* showed that GABA-ergic neurons regulate the phase of the circadian biological clock through pre and postsynaptic mechanisms (1). However, in our study in which baclofen was injected intraperitoneally, our data does not show similar findings (Table 1,2). MC and baclofen when administered separately did not significantly change leptin levels (Table 4). However, baclofen when given to the MC implanted rats lowered leptin levels despite in significant body weight change in this group. This fact can be explained by the possible decrease in gastric leptin release due to the insensitivity of mechanoreceptors in the stomach wall. Both exo- and endogenous CCK inhibits food intake in rats, what can be reversed by selective CCK antagonists (21). Visceral sensory signals detected by receptors are sent to the nucleus tractus solitarius (NTS) *via* vagus nerves (22). NTS remains in a functional relationship with the arcuate nucleus, which has been involved in the control of feeding behaviour determined by metabolic requirements. Information collected from two channels, metabolic and viscerosensory, converges in the hypothalamus. Vagal nerves seem to be critical for the regulation of short-term food intake (23,24). Most of the vagal hypothalamic terminations in the anterior

piriform cortex areas present GABA(B) receptors (3). GABA(B) receptors agonists shows different peripheral and central effects on food intake. Ebenezer showed that intracerebroventricular administration of baclofen increases food intake in satiated pigs (25). GABA (B) receptor agonist-baclofen when given peripherally, induces desensitization and inhibits transient lower esophageal sphincter relaxation and attenuates the suppressant effect of administration of cholecystinin (CCK) on food intake in rats (26,27). In our studies, similar effects of baclofen were observed in rats with chronic stimulation of vagal nerves. Electrical stimulation of the vagal afferents is known to release stored CCK-8 evoking pancreatic exocrine secretion and insulin release (16). This may also explain decrease of glucose levels observed in our experiment in MC implanted rats. Partosoedarso *et al.* proved that GABA(B) receptors agonists inhibit peripherally gastric mechanosensitivity, but not chemosensitivity of vagal afferents (28). Mechanosensory input to brain stem neurons is also reduced centrally by GABA(B) receptors but chemosensory input is unaffected (29). Therefore, the observed attenuation effect of baclofen in our experiments of MC action on the body weight may be related to decreased gastric mechanosensitivity. However, this cannot be attributed to signals which reduce feeding and reach the central nervous system *via* splanchnic nerves (30). Our data is in agreement with those published by Schwartz *et al.* They showed that the vagus nerve seems not to be merely a passive conductor, but also has the capacity to integrate information arising from complex nutrient stimuli (31-33). The vagal nerve is involved in gastrointestinal motility modulation. The central stimulation of vagal nuclei (34-36) or peripheral stimulation (37,10) of the vagal fibers induces changes in gastric motility. The pattern of pressure changes under influence of vagal electrical stimulation in particular parts of the stomach as well as in remaining gastrointestinal is complex and depends on the stimulation type (38-40) and may improve motility or even evokes hypermotility. Our previous studies showed stimulatory effect of direct peripheral stimulation of vagal nerve on gastric motility. On the other hand gastric pacing is known to restore regular slow wave and improve gastric emptying and motility (41). On the basis of these reports we hypothesized that microchip stimulation seems to cause regular hypermotility state and this way decreases nutrient absorption. On the contrary baclofen disrupted regular motility pattern and decreased effect of MC stimulation. This interference of baclofen on body weight in MC stimulated rats suggests GABA-B receptors depended effect of permanent vagal nerve stimulation. The mechanism of final effect of peripheral vagal nerve stimulation on animal feeding behaviour still remains unclear. Our results suggest that microchip vagal neuromodulation through increased vagal afferent activity induces an alteration in the feeding behaviour and decreases nocturnal food intake and body weight. These effects were partially attenuated by baclofen, however daily changes in the food intake pattern were affected but not abolished. Summing up, our data suggests that GABA(B) receptors play an important role

in pathomechanism of attenuation of the food intake induced by vagal nerve stimulation.

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