EFFECTS OF CONTINUOUS MICROCHIP (MC) VAGAL NEUROMODULATION ON GASTROINTESTINAL FUNCTION IN RATS

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Afferent fibers from gastrointestinal tract outnumber efferents ten times in vagal nerves. Modifying the afferent input makes possible to change discharge of vagal efferents affecting gastrointestinal functions in process known as neuromodulation (NM). Lately it has been used in the treatment of pain and hyperactive neurogenic bladder in urology. MC induced NM may therefore provide a concurrent to pharmacology tool, in treatment of gastrointestinal disorders. The aim of this study was to investigate the effects of long term neuromodulation procedure with use of MC on gastric motility, secretion and weight control in conscious rats. Experiments were performed on 30 Wistar male rats (250—350 g) divided in two groups: sham operated and microsurgically implanted with MC on left vagal nerve below diaphragm. Following stimulation parameters were used: frequency of 0.5—30 Hz, amplitude of 0.55 V, impulse duration of 10 ms in monophasic fashion. In both groups food intake and body weight were measured through the period of 2 weeks after recovery period. Then gastric fistula was implanted in gastric antrum and fasted gastric motility recorded with use of PowerLab system (Australia). Gastric emptying and secretion were also tested with use of phenol red and automatic titration methods. On the daily basis glucose level with standard test and leptin after MC implantation were measured. Recording of vagal activity in fasted rats showed burst of action potentials about 5 ± 2.5 in period of 5000 sec, each burst with spike frequency up to 35 Hz. Food (5 ml of Intralipid – intragastrically) almost doubled amount of bursts to 12 ± 5 in period of 5000 sec with increase in frequency at spike up to 50 Hz. MC induced vagal activity showed continuous spike activity similar to fed pattern. MC induced NM decreases daily food intake by 6% (33.6 ± 4.8 vs control 35.5 ± 4.8 g, p < 0.01). Body weight gain in rats before MC implantation decreased by 20% within 2 weeks after recovery (34.8 ± 9.08 vs control 23.56 ± 4.15 g). Fasting control glucose level also decreased of 5.5% (93.15 ± 9.3 vs control 98.5 ± 11.2 mg%, p < 0.05). Frequency of gastric contractions did not change significantly in MC versus control but amplitude of contractions increased of about 66.7% (2.0 ± 0.8 vs 1.17 ± 0.35, p < 0.05) at the frequency 0.08 Hz range and about 71.5% (1.17 ± 0.35 vs 0.68 ± 0.47, p < 0.05) at the frequency 0.12 Hz. in FFT analysis PowerLab (chart v = 4.01). BAO decreased by 29.25% without H+ concentration changes (0.2 ± 0.14 vs 0.14 ± 0.12 mmol/30min, p < 0.05) but MAO did not change in MC rats (0.37 ± 0.25 vs 0.42 ± 0.28 mmol/30min, p>0.05). Gastric emptying of isotonic solution increased by 10% (90.46 ± 5.34 vs 80.39 ± 9.95) percent of marker passing to duodenum /5min,
p < 0.0001). Our results suggest that MC induced NM affect brain-gut axis via influencing metabolic and gastric function and decreases body weight.

**Key words:** vagal nerve stimulation, vagal nerve recording, neuromodulation, brain-gut axis, microchip

**INTRODUCTION**

Implantable electronic devices have found the solid place in medical treatment of some diseases. Following the advances of electronics these biologic stimulators became very small, “intelligent” and well tolerated by the organism. Actually the practical application of selective stimulation in gastrointestinal system is analysed. Enormous amount of bites flow along the neural pathway carrying information from digestive system to the brain. Modifying the afferent input makes possible to change discharge of vagal efferents affecting gastrointestinal functions in process known as neuromodulation (NM). Lately the similar idea has been used in the treatment of pain and hyperactive neurogenic bladder in urology. Microchip (MC) induced NM may therefore provide a new tool in treatment of obesity and functional gastrointestinal disorders. The idea of our study is to give an artificial signal from the stomach to the brain carrying a “message” that stomach is full and brings satiated feeling. In order to fit the exact afferent signal recording vagus nerve activity during fasting and after feeding is necessary. Such intervention in vagal activity is aimed to change alimentary behaviour and reduce the consumption of food in obese patients.

The aim of this study was to investigate the effects of long term NM procedure with use of *in vivo* implantable MC device on vagal activity, gastric motility, secretion and weight control in rats.

**MATERIALS AND METHODS**

Male Wistar rats weighing 250—350 g were housed in separated cages at a room temperature (22 ± 2°C) and on a daylight cycle. Standard laboratory food (Labofeed B, Poland) and tap water were provided ad libitum. Male Wistar rats were anaesthetised with pentobarbital (Vetbutal 0.25 mg/kg i.p. Biowet, Puławy). Jagiellonian University Bioethical Committee approved care and use of the animals. (Grant: 6 PO5C 024 20)

In pilot experiment vagal cervical nerves activity were recorded (PowerLab Amplifieres) in fed, 16 hours fasted rats and MC-stimulated rats. We used cuff electrodes as previously described (1). Recorded data underwent mathematical analysis (FFT). Following experiments were performed on 30 Wistar male rats (250—350g) divided in two groups: sham operated and microsurgically
implanted with MC on left vagus nerve below diaphragm marking electrode appropriately as upper-cathode and lower-anode in the distance of about 0.5 cm. At least two weeks were allowed for recovery before commencing experiments. Following stimulation parameters were used: frequency of 0.5—30 Hz, amplitude of 0.55 V, impulse duration of 10 ms in monophasic fashion. In both groups food intake and body weight were measured through the period of 2 weeks after recovery. On the daily basis glucose level with standard test and leptin before and after MC implantation were measured. Then gastric fistula was implanted in fundus. Rats were allowed to recovery again for at least a week after surgery. Intragastric pressure recording (IGP) were done after overnight fasting (16 hours) (PowerLab system — Australia). Gastric emptying was measured by dye-dilution technique. Phenol red was added to isotonic solution as a non-absorbable dilution marker. Gastric emptying was determined from the volume and phenol red concentration of fluid recovered from cannula after 5 minute. The absorbance of sample was red at a wavelength of 560 nm with Zeiss (Germany) spectrophotometer. The gastric emptying was calculated according to published formula (2, 3). BAO and MAO (pentagastrin stimulation in dose 0.25 μg/kg i.v.) were estimated from collected gastric juice for 30 minutes. Finally vagal nerves activity in control and MC rats were recorded. Results were expressed as mean ± S.D. Statistical analysis was performed using “t” test. Differences were considered significant if the p value was less than 0.05.

RESULTS

Food intake and body weight

MC induced NM decreases daily food intake by 6% (33.6 ± 4.8 vs control 35.5 ± 4.8 g, p < 0.01). Body weight gain in rats during 2 weeks after MC implantation decreased by 20% (23.56 ± 4.15 g vs control 34.8 ± 9.08; p <0.05), and fasting control glucose level also decreased of 5.5% (93.15 ± 9.3 vs control 98.5 ± 11.2 mg%, p < 0.05) (Figs 1 and 2). Leptin serum level remained unchanged (0.7 ± 0.3 vs control 0.6 ± 0.4; p > 0.05).

Gastric motility and secretion

Frequency of gastric contractions did not change significantly in MC versus control rats but amplitude of contractions in MC rats increased of about 66.7% (2.0 ± 0.8 vs 1.17 ± 0.52) at the dominant frequency 0.08 Hz range and about
71.5% (1.17 ± 0.35 vs 0.68 ± 0.47, p < 0.05) at the frequency 0.12 Hz. in FFT analysis PowerLab (chart v = 4.01). (Fig. 3).

BAO decreased by 29.25% without H+ concentration changes (0.2 ± 0.14 vs 0.14 ± 0.12 mmol/30min, p < 0.05) but MAO did not change in MC rats (0.37 ± 0.25 vs 0.42 ± 0.28 mmol/30min, p > 0.05) with increase in H+ concentration. Gastric emptying of isotonic solutions increased by 10% (Fig. 4) (90.46 ± 5.34 vs 80.39 ± 9.95 percent of marker passing to duodenum /5min, p < 0.01).
Vagal activity

Recording of vagal activity in fasted rats showed burst of action potentials about $5 \pm 2.5/5000$ sec, each burst with spike frequency up to $35$ Hz (Fig. 5, 8). Food (5 ml of Intralipid — intragastrically) almost doubled amount of bursts to $12 \pm 5$ in period of 5000 sec with increase in frequency at spike up to $50$ Hz (Fig. 6, 9). MC induced vagal activity showed continuous spike activity similar to fed pattern (Fig. 7).
Fig. 5. FFT analysis of spikes activity in vagal nerves in fasted rats.

Fig. 6. FFT analysis of spikes activity in vagal nerves in fed rats.

Fig. 7. FFT of MC induced spike activity in vagal nerves in MC-stimulated rats.
DISCUSSION

The relationship between size of administered meal and satiety centre has been previously described. Administration of nutrients or mechanical distension suppresses dose-dependently food intake (4). The presence of nutrients in the stomach, its quantity and quality activate neural and humoral signals. The meal-activated reflexes play the important role in regulation of satiety centre. Vagal nerves and splanchnic mesenteric nerves are the peripheral neural components of the brain-gut axis. Afferent fibers from gastrointestinal tract outnumber efferents ten times in vagus nerves of the rat. Afferent fibers of nerve X among others travel impulsion from mechanoreceptors elicited by either passive distention of the gut wall or active stomach contractions. Grundy and Scratchered described three types of receptors: mucosal “touch” receptors, muscular tension receptors and serosal receptors in the stomach. (5). The so-called in series tension receptor are slowly adapting and activation causes discharge in a distension-sensitive afferents. The mucosal receptors are low threshold and respond to different chemicals with fast adaptation to permanent stimulation. Chemical, mucosal receptors are activated by: glucose, aminoacids, fatty acids. Afferent fibers innervating the corpus respond early to gastric filling. Antral contractions generate a burst impulsion. Signals generated in vagal afferents may potentially affect the whole organism. Except satiety data afferents carry information of: nausea, vomiting, pain, fever related to infection.

Vagal efferents have a projection field in the central nervous system called dorsal vagal complex (DVC) and afferents in nucleus tractus solitarius (NTS).
The alternative parallel meal related signaling system are the peptides realised to the blood. These peptides mediate signals through classic hormonal route to the target brain cells. It is actually known that some of them also act via receptors of vagal peripheral termination (vagal afferents) in the gut (6). The example is CCK — physiological hormonal regulator of pancreatic secretion, gastric emptying and food intake. In this respect CCK action via CCKA and CCKB receptors found on the vagal nerves and in the brain may play a main role in inducing gastrointestinal reflexes (7). The response to CCK action on afferent vagal activity is completely absent after truncal vagotomy in rats(8).

Another potent food intake modulator seems to be leptin (9). Exogenous leptin administration inhibits food intake and causes increased energy expenditure in rats (10) and lack of leptin observed in long term fasted obese mice may be responsible of permanent hunger feeling (11). It has been proposed lately that local production of leptin in the stomach acting via vagal afferents take part in short-term satiety regulation (12, 13). Leptin may also interact with insulin and glucocorticoids — hormones associated with obesity (14).

Presented results indicate that low frequency and voltage electrical neuromodulation of vagal discharge influences food intake. The mechanism of it remains unclear. Continuous stimulation with MC induces depolarisation, which blocks all naturally occuring impulsation. Vagus nerve upon electrical stimulation releases stored CCK-8 evoking pancreatic exocrine secretion and insulin release (15). Both exogenous and released endogenously CCK inhibit food intake and this effect is reversible by selective CCK agonists in rats (16). Moreover, chronic administration of CCK antagonists has been reported to increase body weight in rats (17). There are only a few studies examining the role of the gastric pacing on food intake. Cigaina and colleagues using long-term antral stimulation obtained reduction food intake in swines. Increase stool production in paced swines suggests faster visceral peristalsis as a vagal response (18).

CCK plays a pivotal role in fat-induced inhibition of gastric emptying and acid secretion (19). In our study we did not observed changes in H+ concentration but gastric emptying rate was considerably increased. Decrease in BAO can be explained by accelerated gastric emptying despite unchanged pH. MAO remains unchanged. Pentagastrin inhibits gastric emptying in dose-dependent manner by increase in tonus of the pylorus (20). Our results showed that low frequency and voltage impulsation generated by MC influence mostly gastric emptying without gastric secretion. This suggests that there is an overdrive of stimulation over the CCK action. Significant influence of MC on gastric emptying without substantial changes in secretion may be explained by different threshold release of neurotransmitters in parasympathetic system upon electrical stimulation. Vagal nerve stimulation with lower frequency (2.5 Hz) evokes also NO production in gastric tissue whereas VIP release to the blood
was observed at higher frequency (10Hz) (21). Putative release of NO upon low frequency and voltage stimulation may be responsive for sustained relaxation of distal stomach causing gastric emptying acceleration observed in our study.

The frequency of stimulation may have influenced different exocitic mechanism and determined mediator type release from nerve terminations. Low-frequency stimulation (2—5 Hz) of myenteric neurons favours acetylcholine and high-frequency favours VIP release involving L-type voltage-sensitive Ca2+ channels and phosphoprotein pattern (22, 23).

Takahashi describes triphasic response of proximal stomach produced by electrical stimulation of vagal trunk (2.5 and 10 Hz): first rapid transient relaxation, second phasic contraction and third delayed prolonged relaxation. He concluded that vagus nerve stimulation evokes NO and VIP release via nicotinic synapses causing different modes of stomach relaxation (21). In our experiment applied long-term MC produced increase in amplitude contractions without noticeable phasic pattern however in our previous records of short-term stimulation such pattern can be seen (24, 25).

In Grundy studies main frequency of vagal nerve activity was in range 0—30 Hz. Recently Grundy and colleges estimate afferent discharge of vagal nerve. The basic activity was about 1.3 Hz and with the distended stomach about 9 Hz (26). Our preliminary data indicate basic activity between 0—35 Hz with no repetitive pattern of discharge. Increase in amplitude contractions may be explained by direct influence on current on gastric nerves and musculature however we did not observed acceleration of contractions.

In conclusion MC induced neuromodulation of vagal nerve activity stimulates amplitude of gastric contractions and emptying. Basal acid output and food intake decreases. MC induces metabolic changes and decreases glucose level however leptin release remains unchanged. Our results suggest that MC induced NM affect brain–gut axis via influencing metabolic and gastric function and decreases body weight.

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