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ENCODING MEAL IN INTEGRATED VAGAL AFFERENT DISCHARGE

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Vagal afferents are integral part of the negative feedback loop induced by constitution and size of food stomach and jejunum. Aim of this study was to assess vagal discharge in response to food and gastric distension in rats. Electrophysiological recordings of vagal afferents in fasted (n=32), fed rats (n=20) and during gastric balloon distension (n=12) were performed. After 60 minutes of fasted nerve recording tube feeding was done. Fasted rats also underwent gastric distension via oesophagus. Vagal afferents discharges were analysed with dual time-amplitude window discriminator. Total vagal afferent discharge in fasted and fed rats revealed 0.3 ± 0.12 vs 0.56 ± 0.22 Hz ($p<0.05$). We observed two distinct discharge patterns: high amplitude low frequency (HALF) and low amplitude high frequency (LAHF). HALF spikes were observed more frequent in fasted than in fed rats (0.05 ± 0.02 vs. 0.03 ± 0.016 Hz ($p<0.05$). Conversely LAHF spikes in fed rats predominated over their occurrence in fasted rats: 0.52 ± 0.2 vs. 0.25 ± 0.12 Hz ($p<0.05$). Left vagal afferents discharge rises with gastric distension of 6, 8 and 10 ml and were: 0.46 ± 0.22 Hz, 0.65 ± 0.31 Hz, 0.86 ± 0.33 Hz ($p<0.05$) respectively. Similar discharge showed right vagal afferents: 0.41 ± 0.08 Hz, 0.51 ± 0.13 Hz and 0.77 ± 0.27 Hz ($p<0.05$) for 6, 8 and 10 ml of distension, respectively. We conclude that interdigestive information from gastrointestinal tract is encoded in high amplitude low frequency of spikes pattern in the vagus nerves.

Key words: *vagal afferents, food, gastric distension, electrophysiological recordings.*

INTRODUCTION

Vagal communication between stomach and caudal brain stem has long been recognised. Multiunit recordings have been made in 70's from a vagal nerve filaments in response to gastric and chemical stimuli to characterise the signal conveyed centrally (1). However, a direct electrophysiological evidence of vagal overall afferent activity is still lacking. The particular sequence of

afferent informations related to regulation of intragastric pressure, gastric accomodation and emptying are transmitted rostrally into the caudal brain stem which contains neuronal mechanisms that may play a role in regulation of the food intake and satiety. Vagus nerve neuromodulation techniques are being currently developed for treatment of obesity and functional GI diseases (2,3). Better recognition of native afferent activity to design natural-like pattern of electrical stimulation would improve effects of vagal stimulation.

Velocity of neural transmission is limited. This suggest that neuromodulation is possible only in the certain frequency range, which can effectively affect activity of neural fibbers. The aim of this study was to evaluate the range and pattern of overall vagal activity in fasted and fed rats and obtain hints for selective vagal stimulation for food intake regulation.

MATERIALS AND METHODS

Male Wistar rats (n=32) weighting 250-350 g, housed at a room temperature (22 ± 2 °C) and 12 hours light/dark cycle were involved into the study. Standard laboratory food (Labofeed B, Poland) and tap water were provided ad libitum. Before every experiment rats were food deprived for 16 hours. Operations were performed under general anaesthesia with pentobarbital (Vetbutal 0.25 mg/kg intra peritonealy Biowet, Poland). Supplemental injection was administered every 20 minutes under control of general condition, heart rate and breathing. Body temperature during surgery was maintained at 36-37 °C with a warm-water heating pad.

The electrophysiological recordings were performed in fasted rats (n=32) and after gastric tube feeding (n=20) or gastric distension using latex balloon (n=12). According to research protocol left or right cervical vagus nerve was isolated on the neck of the rat and microsurgically dissected from surrounding tissues and perineurium. Vagus nerve was transsected and the cuff electrode was placed on its peripheral, afferent end (4). Latency period of 30 minutes before commencing of the recording was required in order to avoid damage response after electrode placement. Sixty minutes of vagus nerves recording was performed in fasted animals and assumed as reference. Than, mixed food (5 ml glucose 5%- 4kcal/1g, 3 ml aminoacids 10%-16gN/L, 2 ml Intralipid 10% 10kcal/1ml) was infused in to the stomach via esophageal catheter. Infusion rate did not exceed 0.3ml/1min in to avoid overactivation of mechanoreceptors. The second group of fasted rats (n=12) after control recordings underwent gastric distension (6, 8 and 10 ml) with latex balloon placed in the stomach via oesophagus. Vagal afferent discharges were amplified using previously described standard technique (PowerLab amplifiers) and stored on PC hard disc.

The collective discharges were analysed with dual time-amplitude windows discriminator software (PowerLab software). Comparison between control and distension groups were calculated using Student's t test ($p<0.05$). Multiple group comparisons were performed by analysis of variance (ANOVA) followed by Fisher's least significant difference test. Ethical Committee of Jagiellonian University (Poland) for animal experiments approved the experiment.

RESULTS

The total vagal afferent discharge frequency in fasted and fed rats was 0.3 ± 0.12 vs 0.56 ± 0.22 Hz ($p<0.05$), respectively (*Fig.1*). We observed two distinct patterns

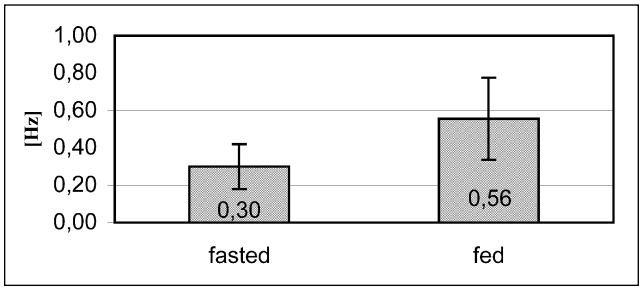

Fig. 1. Total vagal afferent discharge in fasted and fed rat.

of the vagal multiunit response: high amplitude low frequency (HALF) and low amplitude high frequency (LAHF). This pattern division was made on the assumption that impulses LAHF were at least two times lower than maximal impulse amplitude observed at a given nerve recording. In fasted rats frequency of HALF spikes potentials predominated over HALF pattern after food administration with frequency of 0.05 ± 0.02 vs. 0.03 ± 0.016 Hz ($p<0.05$), respectively. Conversely frequency of LAHF spikes potentials in fed rats predominated over fasted rats with frequency of 0.52 ± 0.2 vs. 0.25 ± 0.12 Hz ($p<0.05$), respectively (*Fig.2,3*). Vagal fed pattern were observed only during first 15 minutes after food administration and then the vagal discharge returned to fasted pattern.

Frequencies of left vagal afferent discharge increased significantly in parallel to gastric distensions with 6,8,10 ml and were: 0.46 ± 0.22 Hz, 0.65 ± 0.31 Hz, 0.86 ± 0.33 Hz ($p<0.05$) respectively. Similar pattern of discharges were obtained from right vagal afferents during gradual distension: 0.41 ± 0.08 Hz, 0.51 ± 0.13 Hz, 0.77 ± 0.27 Hz ($p<0.05$) respectively (*fig. 4,5*).

DISCUSSION

Afferent fibbers of vagal nerves are integral part of the brain-gut axis, which is involved in negative feedback loop related to food presence in gastrointestinal

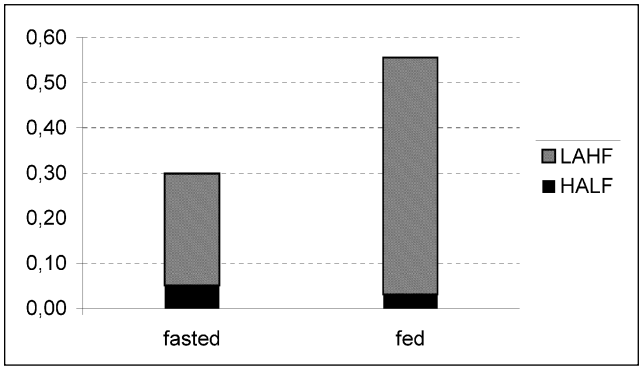

Fig. 2. HALF and LAHF pattern in fasted and fed rat.

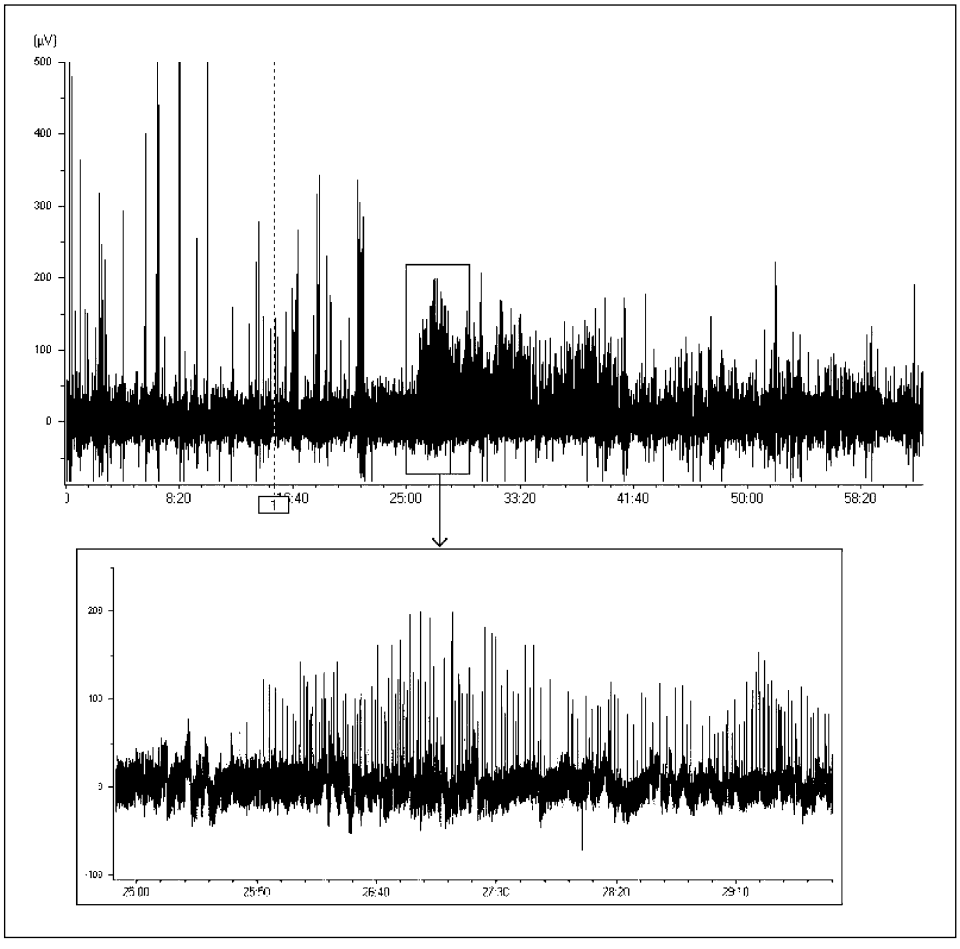

Fig.3 The example of the original vagal afferents recording before and after the meal injection.

tract (5). The vagal afferent system detects gastrointestinal events in the periphery and generates appropriate behavioural, autonomic and endocrine response. The nerve itself is merely a cable receiving and conducting information in shape of electrical impulses from peripheral interface to its central interface in the brain (6,7,8). Schwartz *at al.* provided data that vagus nerve also has the capacity to integrate information arising from complex nutrient stimuli (9). Vagus nerve seems to be a target for regulation of food intake and body weight using proper permanent electrical stimuli (10).

The vagal nerve endings in upper GI tract are sensitive to various physical and chemical stimuli (11,12). Vagal mechanoreceptors in the gut muscular layer are spontaneously active with irregular low-frequency discharge. This activity probably plays a role in the monitoring of resting gastrointestinal muscle tension

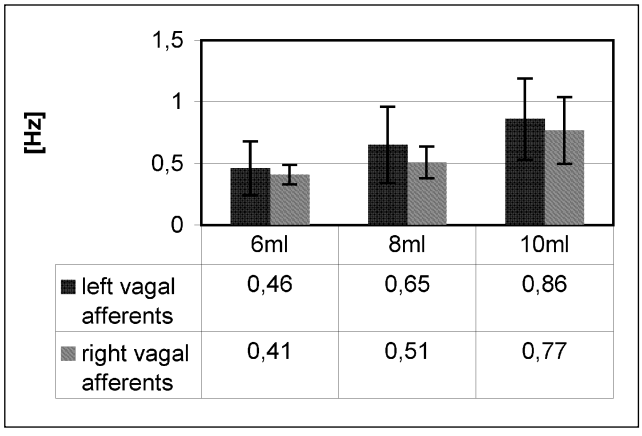

Fig.4. The vagal overall afferent discharge upon different gastric distension.

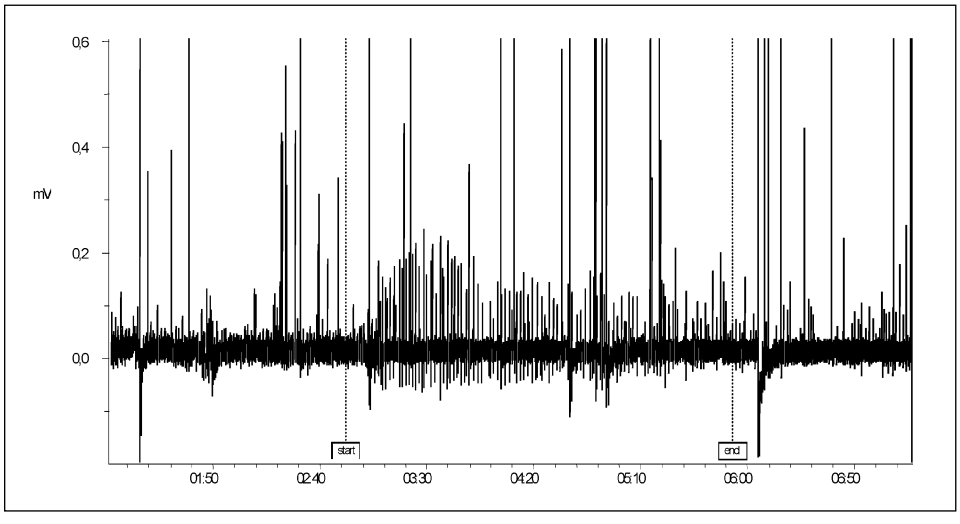

Fig.5 Vagal overall afferents recording during gastric distension (8ml).

(13). In fasted rats we observed single and multiple spikes with high amplitude, which may result from phase III of motor migrating complex (MMC) present during fasting and creating highest increases in intraluminal pressures. Similar to Mei (12) who shown that duodenal endings sensitive to chemical stimuli are spontaneously inactive and react in "on / off " manner, we observed that mixed food elicits "on" reaction with low amplitude spikes activity in vagal afferents, which lasts only 15 minutes, and then returns to fasted pattern. This period equals gastric emptying time of liquids. Spontaneous vagal afferents activity was similar to frequency range recorded by other authors. Vagal discharge captured at esophageal level of rat was 1-15 Hz (14) whereas in dogs was 0.2-13 Hz (15). Nerve activity captured from the same area in the cats in two different studies was

in the range of about 5-30 Hz and 1-25 Hz, respectively (16,17). These discrepancies most likely result from different technique of recordings and preparation and electrodes used. In our study we recorded discharge activity in the range 0.3-0.56 Hz. Fasted and fed pattern were strongly differentiated by frequency range. Barber *at al.* (18) studying brain responses to electrical stimulation of ventral vagal gastric fibers have shown that brain stem unitary discharge was unable to follow stimulus frequency of 20 Hz or greater. Our data extend observation from previous studies showing that vagal input from the stomach code dynamic and static changes in response to gastric distension.

The presence of food in duodenum elicits specific postprandial motility and activates vago-vagal reflexes to stimulate pancreatic secretion being accompanied by the fall in plasma ghrelin and increase of plasma leptin, reflecting feeding behaviour (19). The chemical content of the food is most likely detected by duodenal nerve endings (chemoreceptors), whereas volume of a meal stimulates mechanoreceptors in upper gut, leading to distinct motility response (20,21). Mathis et al. have reported that predominant discharge is coming from load sensitive mechanoreceptors of the rat stomach (22). Ozaki *et al.* indicated that gastric volume stronger than nutrient content inhibits food intake in rats (23). We confirmed these data by showing that most intensive vagal response resulted from mechanical distension is induced by food intake. However, there are significant differences in vagal activity related to chemical composition of given food (20). Thus, it seems logical and possible to hypothesize that not only size but also meal component influence food intake and all this information is partially encoded in vagal afferent discharge beside the humoral signals (24). The presence of chemically distinct food in the duodenum elicits clearly different afferent discharge in the vagus nerve (12). The presence of unique chemoreceptors in the rat stomach was not proved in our study. The gastric sensory units are mostly polymodal in the rat as well as in some other species (25,26). However expression of bitter taste receptors of the T2R family in the gastrointestinal tract has been reported not only in duodenum, but also in the stomach (27). The presence of protein G: α-gustducin in taste cells is associated with induction of taste signal. These facts may indicate potential possibility of gastric chemoreception to affect food intake (28). Our major finding is that two different pattern of vagal overall discharge are induced by food or gastric distension and those after meal are short lasting. This suggest involving both signals from mechano- and chemoreceptors confirmed by our previous studies (29,30,31). Products of trigliceride digestion given to the rat stomach inhibits food intake via release of CCK, which in turn act on vagus nerve sensory endings (32). The meaning of hepatic glucoreception in food intake regulation seems to be minor. The alimentary behaviour in rats after surgical liver vagal denervation remains unchanged (33). In summary, food intake acts via neurohumoral rout of the brain-gut axis and vagally mediated food induced negative satiety feedback loop acts via the following sequence of events:

size of meal in upper GI tract (mechanoreceptors), chemical content in duodenum (chemoreceptors) and caloric load in liver (glucoreceptors).

We conclude that sensory information collected form peripheral mechanoreceptors is encoded as combined amplitude-frequency and sequence spikes pattern in the vagus nerves only after short postprandial period.

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