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THE VAGAL AFFERENTS DISCHARGE AND MYOELECTRICAL ACTIVITY IN THE GASTRIC HYPERALGESIA MODEL IN RATS

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A long term exposure of the gastric mucosa to inflammatory factors is suspected to alter the normal stomach motility. The consequence of it is an abnormal sensomotor response to food causing dyspeptic symptoms. Our study aimed to investigate the vagal afferents activity and the gastro-duodenal slow wave response to the mild gastric mucosa inflammation in rats. The gastric mucosal inflammation was induced by addition iodoacetamide to drinking water for 5 days. The gastro-duodenal slow wave, vagal nerve recordings and the gastric mucosa examination were performed on 6th day. The iodoacetamide irritated gastric mucosa presented the minimal inflammatory infiltration with mast cells. The vagal afferent activity was significantly increased after iodoacetamide treatment from 0.3 ± 0.1 to 1.9 ± 0.58 Hz, ($p < 0.05$). The gastric slow wave accurate frequencies extracted from the fast Fourier transform spectra accelerated from 0.08 ± 0.01 to 0.1 ± 0.02 Hz ($p < 0.05$). The duodenal frequencies remained unchanged (from 0.64 ± 0.02 to 0.59 ± 0.1 Hz). These results suggest that mild gastric mucosa irritation sensitizes vagal afferents and alters gastric but not duodenal pacemaker activity which may contribute to dyspeptic sensations.

Key words: *iodoacetamide, vagal afferents activity, gastro-duodenal slow wave*

Abbreviations: *iodoacetamide (IA), fast Fourier transform (FFT), interstitial cells of Cajal (ICC)*

INTRODUCTION

The inflammation has been proposed to induce the visceral hyperalgesia leading to development of functional diseases of the GI tract (1, 2). A long term

exposure of gastric mucosa to inflammatory factors is suspected to alter the normal stomach motility (3). The consequence of it is an abnormal sensomotor response to food leading to development of dyspeptic symptoms (2). The underlying mechanism of inflammation induced dysmotilities seems not to have a universal character and the slow wave abnormalities can be a common final consequence. Recent reports indicate the significant role of subacute inflammatory process in the GI tract contributing to chronic functional dyspeptic syndrome in humans. Many papers focus on particular inflammatory molecules as main candidates responsible for dyspeptic symptoms. The inflammation usually develops in universal sequences but the chronic effect depends on the tissue ability to self limitation of this process. This might suggest that genetic predisposition relevant to cutting inflammatory process may play a crucial role. The insidious inflammatory process in the GI tract is very common and often maintained by the presence of viral or bacterial pathogens and because of subacute character the process of restriction may never be initiated. The gastrointestinal motility is determined by the neurohumoral mechanisms where the crucial roles play vago-vagal reflexes, Cajal cells and the enteric nervous system. Considering that our study aimed to investigate vagal afferents activity and gastro-duodenal slow wave response to mild gastric mucosa inflammation in rats.

MATERIAL AND METHODS

Rats (n=35) received 0.1% iodoacetamide (IA) in drinking water for the 5 consecutive days (1). Slow wave recordings were performed before iodoacetamide treatment and on the 6th day in the same group of rats (n=15). The vagal nerve recordings were performed according to the same protocol but in different groups: control (n=10) and iodoacetamide pretreated group (n=10). The control group received 0.9 % saline injection according to the same protocol. This study was approved by the local Ethics Committee of Jagiellonian University Medical College.

Myoelectric activity

A single pair of unipolar silver electrodes were implanted on the distal stomach and proximal duodenum and tunneled on the neck of the rat. After surgery rats were allowed to recovery for 5 days. The slow waves were captured from the conscious, 14 hours fasted rats. The electrodes were connected to the amplifier and captured signal was filtered with the digital band pass filter 0,01-1Hz. The modified FFT (Fast Fourier Transform) spectrum analysis of 1 hour recordings for each animal was performed. The accurate dominant frequencies were read off from the FFT spectra and interpreted respectively as the gastric or the duodenal basic electrical rhythm (4).

Vagal nerve recording method

Vagal recordings were performed in anaesthetized fasted rats. The cuff electrode was placed on isolated afferent cut end of the cervical left vagus nerve in control and iodoacetamide treated rats. Recordings started 15 minutes after the cuff electrode placement and lasted 1 hour. Mass vagal discharges were amplified, recorded on hard disc then filtrated and analyzed with mathematical tools (Power Lab) (5).

Histological studies

In all experimental groups after performed recordings rats were sacrificed and stomach was taken for histological analysis. The paraffin embedded sections were prepared and the specimens were stained with hematoxylin – eosin for the routine microscopy and with toluidine blue for the mast cell evaluation. The mast cell number was assessed in 10 fields with magnification 200x.

Statistics

Statistical analyses were performed using a paired and unpaired t-test and values of $p < 0.05$ were regarded as significant. Data were presented as the mean \pm standard deviation.

RESULTS:

Vagal activity and gastro-duodenal slow wave

The vagal afferent activity was significantly increased after iodoacetamide treatment from the baseline value of 0.4 ± 0.1 to 1.9 ± 0.58 Hz ($p < 0.05$) (*Fig. 1*). The gastric slow wave frequency accelerated from the baseline value of 0.08 ± 0.01 to 0.1 ± 0.02 Hz ($p < 0.05$). The duodenal dominant slow wave frequency remained unchanged (from the baseline value of 0.64 ± 0.02 to 0.59 ± 0.1 Hz). *Fig. 2* and *3* present values of dominant frequencies extracted from FFT spectrum either vagal afferents activity or the slow wave respectively. The rat ECG (about

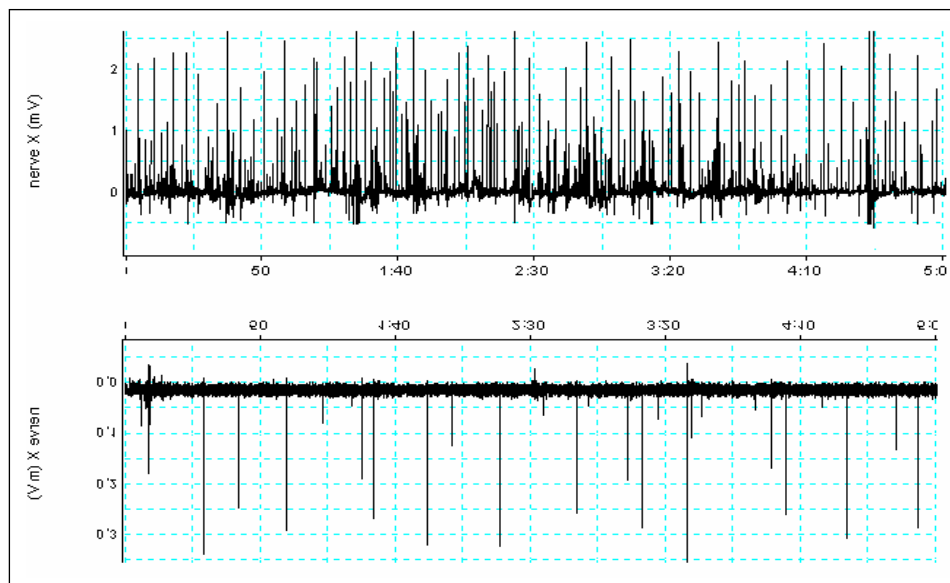


Fig. 1 The vagal afferents discharge in response to the gastric mucosa iodoacetamide irritation pretreatment versus control vagal afferents discharge (5 min recording)

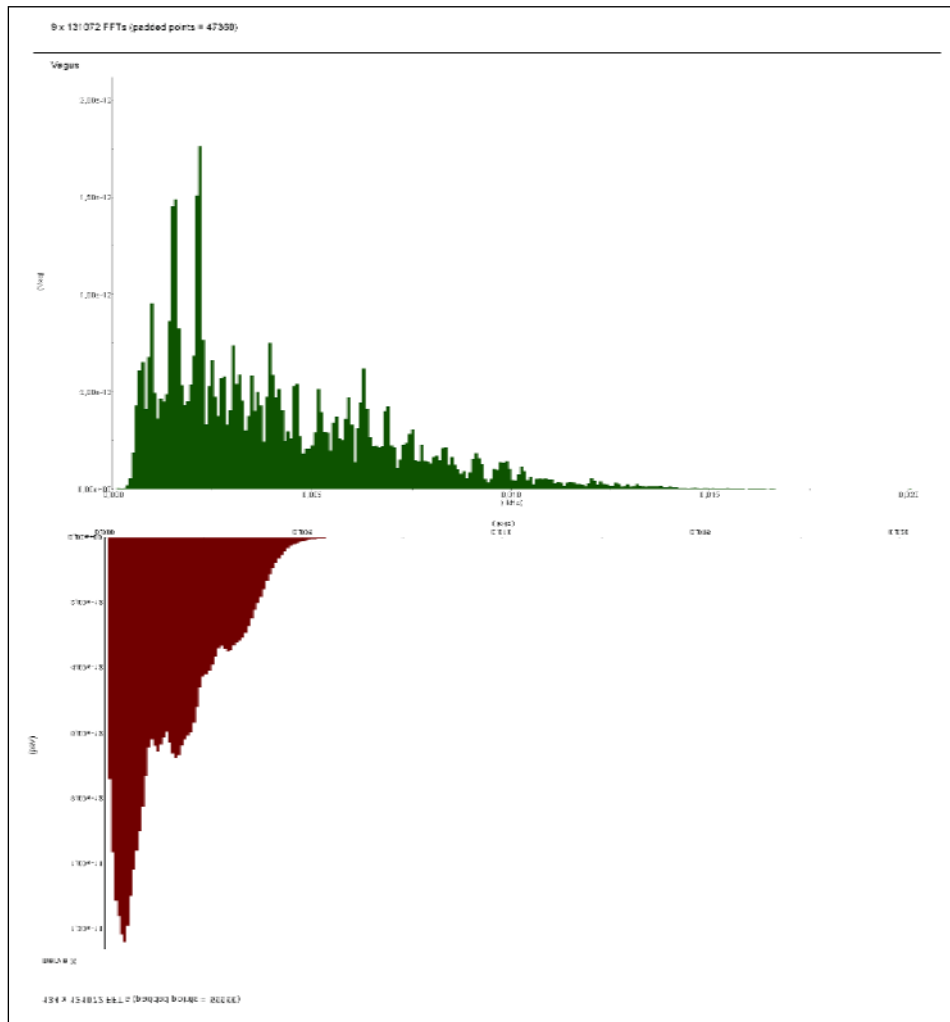


Fig. 2 The FFT spectra of vagal nerve recording in response to the iodoacetamide treatment versus control

5 Hz) and respiration rate (about 1 Hz) during experiment showed no relationship with the slow wave frequency (Fig. 4).

Histological changes:

No macroscopic lesions were observed in the stomach of iodoacetamide treated rats. Microscopically iodoacetamide irritated gastric mucosa and caused the minimal inflammatory changes with the moderate thickening of the submucosal layer, the predominant submucosal mast cells infiltration,

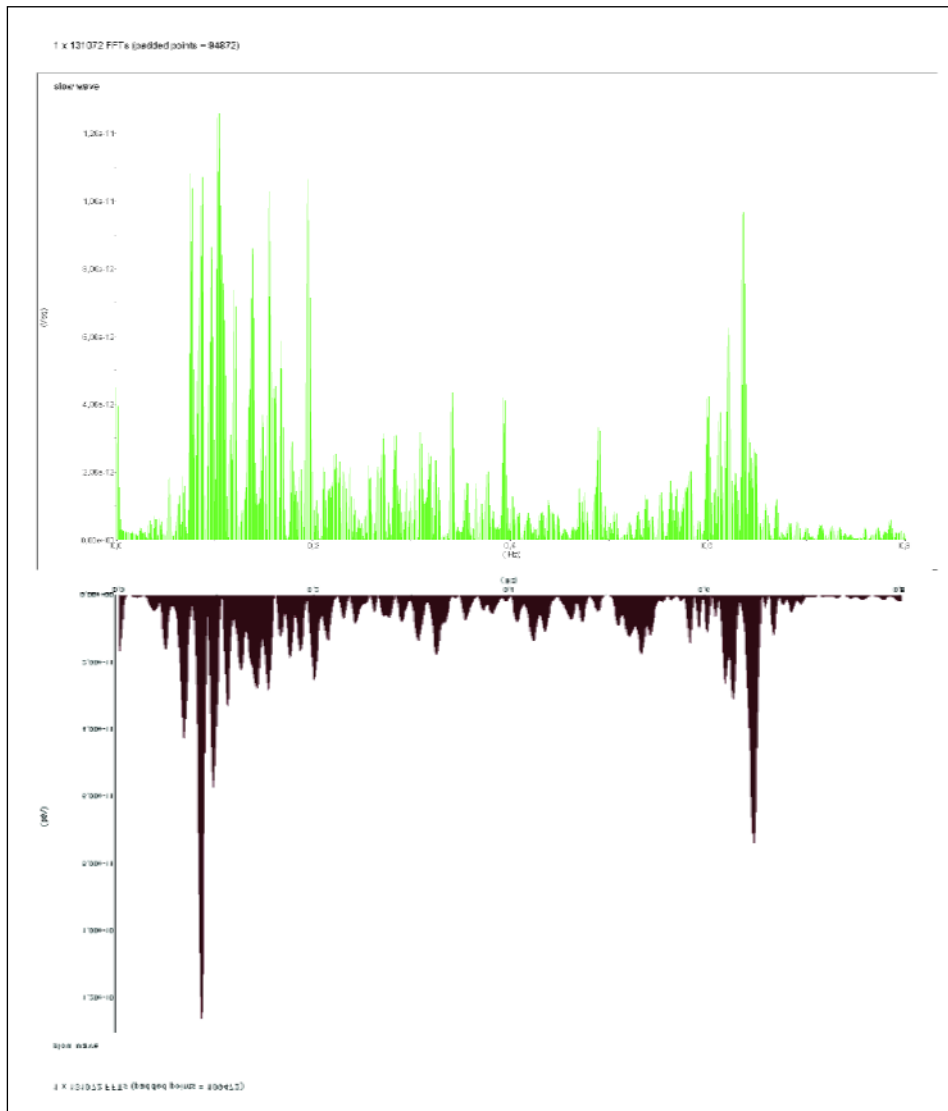


Fig. 3 The FFT spectra of the slow wave recording in response to the iodoacetamide treatment versus control

containing also some neutrophils and lymphocytes (*Fig. 5*). Those changes were especially visible in the antrum in multiple sections. No histological changes associated to iodoacetamide treatment were observed in other layers of the gastric wall.

The total mast cell number in the gastric wall increased in IA group (208.4 ± 53.6) in compare to control (156.6 ± 42.2) and was statistically significant

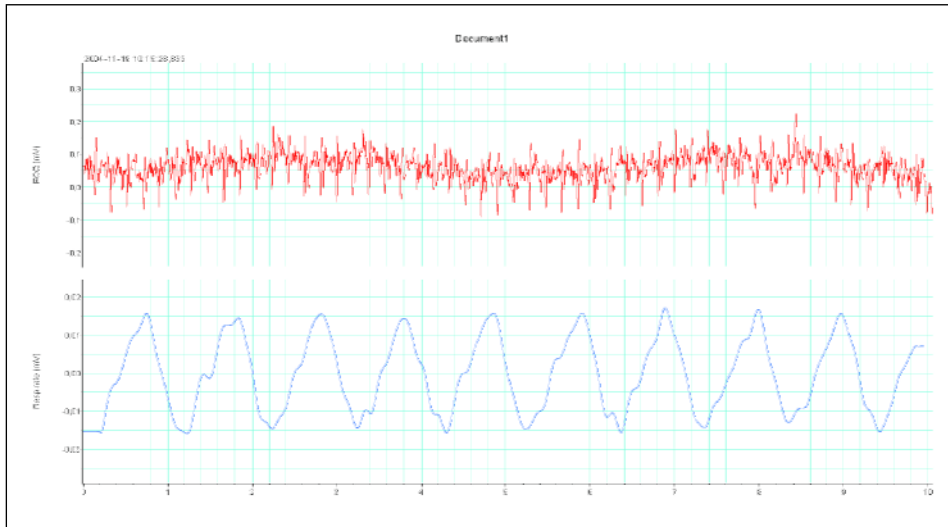


Fig. 4 The rat ECG (about 5 Hz) and respiration rate (about 1 Hz) during the experiment showed no relationship with the gastrointestinal slow wave frequencies.

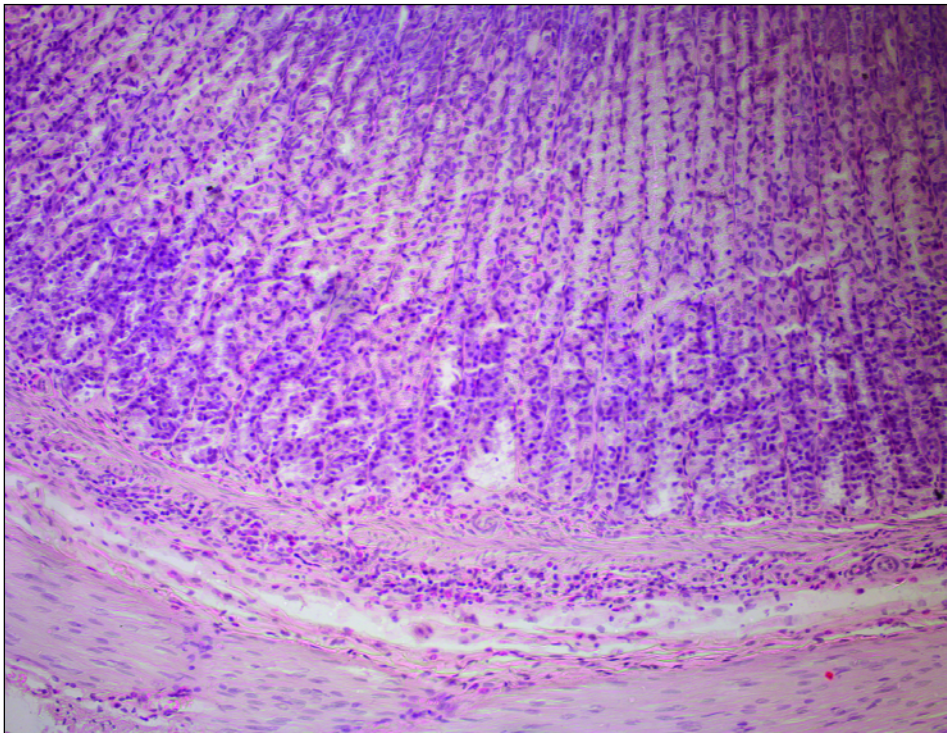


Fig. 5 The gastric mucosa. Mild inflammatory infiltrations in the mucosa and the submucosa. Hematoxylin - eosin staining. Magnification 200x

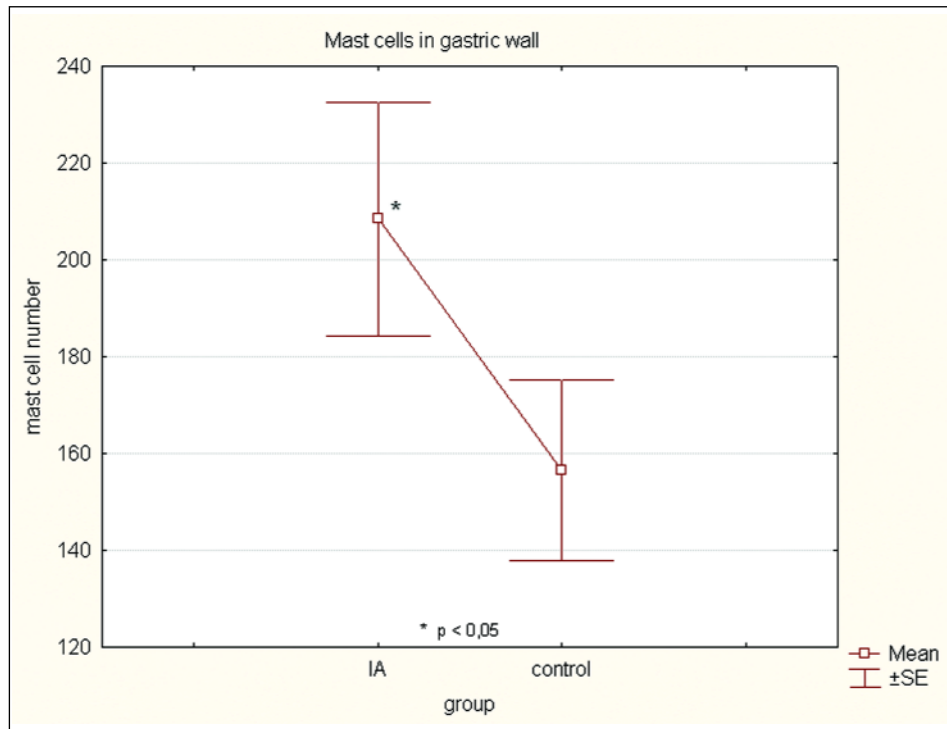


Fig. 6. The mast cells number in the gastric wall in control and iodoacetamide treated rats. ($p < 0,05$)

($p < 0,05$) (Fig. 6). Mast cells were predominantly localized in the submucosa and were partially degranulated (Fig. 7).

DISCUSSION

Usefulness of iodoacetamide in inducing gastric hyperalgesia in rats has been reported by Ozaki *et al.* (1). IA is a sulfhydryl blocker and reduction of this substance in gastric mucosa affects its integrity and causes oxidative damage. IA treatment increased mucosal PGE₂ content with apparent expression of COX-2 mRNA in the stomach (6). The inflammatory cytokines generated during this process are believed to sensitize the primary afferent fibers and the complex chain reaction leads to prolonged hyperalgesia and visceral hypersensitivity (2). IA model of the gastritis has the neurogenic inflammation character and involves capsaicin-sensitive afferent nerves (7). We used the vagal afferent discharge as the marker of visceral hypersensitivity. Our results showed the increased vagal afferent discharge in response to the IA treatment. Similarly, previous studies on gastric ulcers reported increased the resting vagal discharge and enhanced

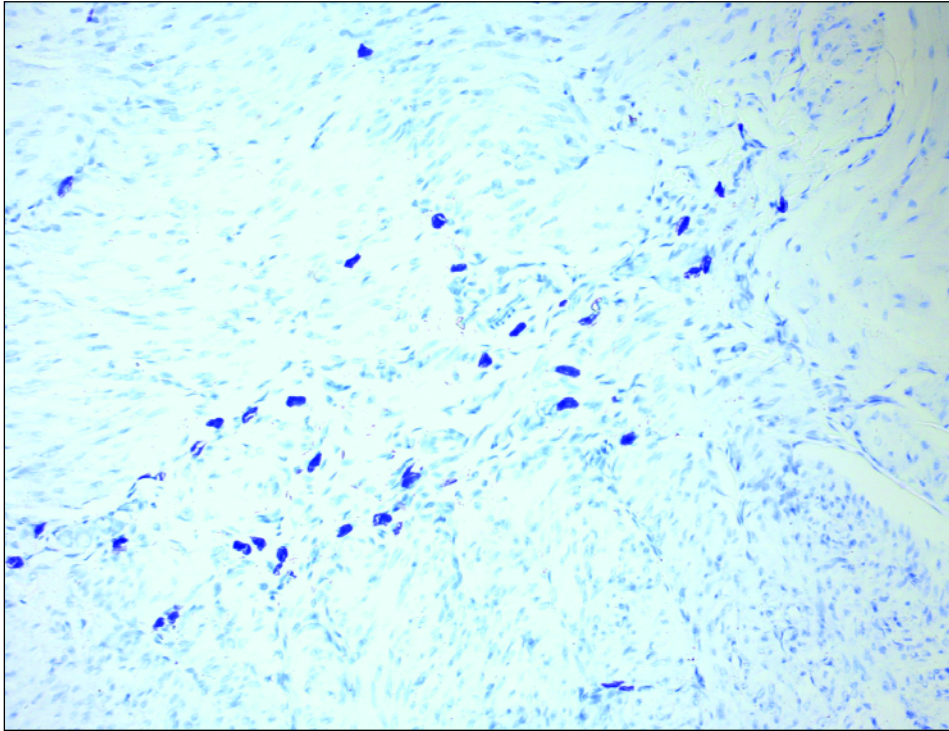


Fig. 7 The mast cells (pink – blue cells) in gastric wall. Toluidine blue staining. Magnification 200x

response to the gastric distension explained by the sensitization in inflamed mucosa (8). Recent studies provided evidence that the local gut inflammation activates vagal sensory neurons (9). This finding correlates with the enhanced visceromotor response to the gastric distension observed in different models of gastric insult (10). The particular cytokines (for example IL-1 or PAF) were found to be a very potent activator of vagal afferents (11, 12). This suggests an important role of vagal nerve in conveying inflammatory information from the gut. Going into details the most abdominal vagal afferents conduct the sensory information in the C-fiber range (13). Capsaicin deafferentation was shown to alleviate inflammatory parameters in experimental IA induced gastritis (7). C-fibers were suggested as crucial in the process of inflammatory hyperalgesia (14). In consequence, inflammation induced changes of the visceral sensation can elicit dyspeptic symptoms (15). We provide the first description of slow wave disturbances by the IA induced mucosal inflammation. This evidence may partially explain the gastric motility disturbances as an effect of persistent inflammation running in mucosal layer. Our findings reinforce the idea that inflammatory mediators induce the excitability of vagal sensory neurons and may contribute to the functional dyspepsia (16). The link between the functional

dyspepsia and the slow wave disorganization was established in different studies (17). In the study of Piqueras *et al.* IA increased the gastric acid secretion response but did not influence the gastric emptying in mice (18). Prior studies have demonstrated some gastric motility abnormalities in gastritis or in response to particular inflammatory mediators (3, 12). The inflammation caused by *Helicobacter pylori* may affect the proximal stomach motility but recent reports are inconsistent (19, 20). A group of researchers: Kang and Bielefeldt have suggested that the stomach inflammation directly affects the gastric sensory and motor function. According to them the inflammation impairs vagovagal reflexes and in consequence changes the gastric motility (3). Thus afferent and efferent pathways both contribute to the development of the dyspeptic symptoms. The outcome of this activation may be the deterioration of the slow wave seen in our gastritis model. The slow wave generators - interstitial cells of Cajal have been found to be active in the mechanosensitive function. Stretching gastric muscles fragments caused ICC membrane depolarization and increased the slow-wave frequency (21). In the inflammation induced hypersensitive state threshold for mechanoreceptors activation is decreased. This evidence provides a link between the hypersensitivity and the slow wave deterioration in the gastritis. In conclusion, we have demonstrated that mild gastric mucosa irritation sensitizes the vagal afferents and alters the gastric but not duodenal pacemaker activity which may contribute to the dyspeptic sensations.

Conflicts of interest statement: None declared.

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