Peptides 31 (2010) 1099–1108

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Endogenous orexin-A modulates gastric motility by peripheral mechanisms in rats

Mehmet Bülbül[a,1], Ruken Tan[a], Burcu Gemici[a], Sebahat Özdem[b], İsmail Üstünel[c], Nuray Acar[c], V. Nimet İzgüt-Uysal[a,*]

[a] Akdeniz University, Faculty of Medicine, Department of Physiology, 07070 Antalya, Turkey
[b] Akdeniz University, Faculty of Medicine, Department of Biochemistry, 07070 Antalya, Turkey
[c] Akdeniz University, Faculty of Medicine, Department of Histology and Embryology, 07070 Antalya, Turkey

ARTICLE INFO

Article history:
Received 8 January 2010
Received in revised form 5 March 2010
Accepted 5 March 2010
Available online 20 March 2010

Keywords:
Fasting
Gastric motility
Orexin-A
Preproorexin

ABSTRACT

Orexin-A (OXA) and orexin receptor type 1 (OX1R) are found in enteric nervous system and smooth muscle cells in the digestive tract. Fasting is a stimulant for OXA synthesis. The aim of the present study was to investigate central and peripheral effects of endogenous OXA on gastric motility. Endogenous OXA synthesis was induced by 36 h fasting. Vagotomy was used to evaluate N.vagus-mediated effects of OXA. Gastric emptying and interdigestive gastric motility were measured by spectrophotometric and manometric methods, respectively. Rats were pretreated with OX1R antagonist SB-334867 prior to measurements. Plasma OXA concentration was assayed with radioimmunoassay while preproorexin (PPO) expression was determined with Western blotting in gastric and hypothalamic tissues. OXA immunoreactivity in antrum was determined with immunohistochemistry. Plasma OXA level, PPO protein expression and OXA immunoreactivity were significantly increased in response to 36 h fasting. Endogenous OXA facilitated gastric emptying and inhibited gastric interdigestive motility. As these effects were abolished with SB-334867, it is likely that gastrokinetic effects of OXA are mediated via OX1R. Vagotomy did not alter OXA-mediated effects. According to current data, OXA is up-regulated both centrally and peripherally upon fasting. Endogenous OXA accelerates gastric emptying while it inhibits interdigestive motility.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

OXA and orexin-B (OXB) also known as hypocretin I and II, are novel neuropeptides which were initially identified from extracts of rat brain in 1998 [11,43]. Both forms are made from preproorexin (PPO) by proteolytic cleavage. Orexins are thought to be involved in diverse functions, such as sleep/awake, feeding behavior, behavioral activity, energy homeostasis and neuro-endocrine responses [21,32,39,43]. Orexins show their effects via OXR1 and orexin receptor type 2 (OXR2) which belong to the family of G-protein-coupled receptors. OX1R has an approximately 10-fold greater affinity for OXA than OXB; while, the OX2R has a similar affinity for the two peptides [Sakurai, 1998 #41, 46].

Within the central nervous system (CNS), orexin-producing cells are located in the lateral and distal hypothalamus. However, the widespread projection of the orexin neurons throughout the neuroaxis, suggesting that activation of orexin circuits probably modulates a variety of systems [26,30]. OXA and OXB nerve fibers project widely into the olfactory bulb, cerebral cortex, thalamus, hypothalamus, and brainstem. Furthermore, dense populations of orexin-containing fibers are present in the paraventricular thalamic nucleus, central gray, raphe nuclei, and locus coeruleus suggesting the roles of orexins in autonomic and neuroendocrine control [10].

The orexin receptors are distributed in a wide range of peripheral tissues including the intestine, pancreas, adrenal gland, kidney, adipose tissue and various regions of alimentary tract [14,23,27,35,37]. Since fasting is the most efficient stimulant for orexin-A production in CNS and gastrointestinal system (GIS), orexin-A release is increased upon nutritional depletion [26,34,43]. Within lateral hypothalamus, OXA-producing cells work as a sensor for the nutritional status, which are activated by hypoglycemia and inhibited by blood glucose [6]. OXA and PPO levels increase in response to 48 h fasting in rats [12,34,43]. During fasting, increased plasma OXA has been shown in human [29] and rat [40].

* Corresponding author. Tel.: +90 242 2496962.
E-mail addresses: mbulbul@mcw.edu (M. Bülbül), nimetu@akdeniz.edu.tr (V.N. İzgüt-Uysal).
[1] Present address: Medical College of Wisconsin, Department of Surgery, Zablocki VA Medical Center, 5000 W. National Ave, Milwaukee, WI 53295, USA. Tel.: +1 414 384 2000/41463.

0196-9781/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2010.03.007

Migrating motor complex (MMC) occurs during the interdigestive state and consists of three distinct phases. Phase I (period of motor quiescence), phase II (period of irregular low-amplitude contractions) and phase III (period of regular high level contractions) [1,55]. The length of the MMC cycle shows variations in different species. The phase III periodically occurs every 90–100 min in humans and dogs; whereas, they are observed every 12–15 min in rats [2,16] and mice [55]. As it is rather difficult to distinguish three phases in rats, these phases are called as phase I-like contractions and phase III-like contractions [16].

The accumulating data suggest central and peripheral actions of OXA on gastric motility. Central OXA administration was shown to stimulate gastric emptying in rats [17]. Since OX1R is highly expressed on dorsal motor nucleus of vagus (DMN), OXA has a potent and long-lasting effect on gastric contractility by stimulating vagal excitatory motor neurons [18,31]. In addition, central OXA administration was shown to increase gastric acid secretion, which was inhibited by vagotomy, suggesting the role of OXA in brain–gut axis [48]. Kobashi examined the effects of centrally administered OXA on gastric motility and found OXA-induced relaxation of the proximal stomach lasting for more than 30 min in rats [28]. In contrast, peripheral administration of OXA showed an inhibitory effect on small intestinal MMC, which was not affected by vagotomy [15,37].

The role of endogenous OXA in gastric interdigestive motility has not been investigated. Furthermore, it is not clear whether OXA modulates gastric motility via peripheral or central mechanisms. The aim of the present study is to elucidate the effects of endogenous OXA on gastric motility in rats. To further investigate this, we induced the endogenous OXA synthesis with long-term fasting in peripheral and central tissues.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 300–350 g were used for the experiments. The animals were housed in temperature and light-controlled rooms (20–22 °C and 12 h day/night cycle) and supplied with standard laboratory chow and water ad libitum. The Animal Care and Use Committee of Akdeniz University approved all experimental procedures involving animals.

2.2. Experimental protocol

Prior to the experiments, the adequate duration of fasting for boosting endogenous OXA was determined with a preliminary study. Food was withheld for 0, 18, 24, 36, 48 or 72 h but with free access to water. During fasting period, coprophagy was prevented by housing animals individually on wire mesh. At the end of the fasting, blood samples were withdrawn and OXA levels were assayed in plasma by radioimmunoassay (RIA). To avoid from circadian changes, all blood samplings were done at 10:00 a.m.

Rats were divided randomly into two main groups for gastric emptying and motility experiments. Eight to ten rats were used in each group. 36 h fasting was used to stimulate endogenous OXA synthesis. In the first set of experiments, the animals for gastric emptying measurement were divided into eight groups as: Fed (no fasting), 18 h fasting (underwent fasting for 18 h), 18 h fasting-SB (underwent fasting 18 h and i.v. administration of SB-334867), 18 h fasting-VG (underwent fasting for 18 h and vagotomy treatment), 36 h fasting (underwent fasting for 36 h), 36 h fasting-VG (underwent fasting for 36 h and vagotomy treatment), 36 h fasting-SB (underwent fasting for 36 h and i.v. administration of SB-334867) and 36 h fasting-VG-SB (underwent fasting for 36 h, vagotomy treatment and i.v. administration of SB-334867). In the second set of experiments for gastric motility recording, animals were divided into same groups except the Fed group. The Fed group animals were allowed ad libitum feeding.

2.3. Animal preparation

Following overnight fasting, rats were anesthetized with intraperitoneally (ip) administered ketamine HCl (90 mg kg^{-1}) and xylasine HCl (10 mg kg^{-1}). A polyethylene catheter (1.27 mm OD) (Harvard Apparatus, Holliston, MA, USA, 59-8330), was inserted through the antrum and fixed at the gastric wall with a purse-string suture. The catheter was run subcutaneously, emerged at the back of the neck and secured to the skin. The catheter was filled with heparinized saline to prevent obstruction. To evaluate the involvement of vagal nerve, vagotomy was performed at the same surgical session. Under a dissecting microscope, the trunks of the subdiaphragmatic vagus were exposed and were dissected from the esophagus. Before experiments, surgical success of the vagotomy was confirmed by the measurement of gastric acid output. After surgery, rats were housed individually with access to standard diet and tap water. Intramuscular injections of tramadol HCl (20 mg kg^{-1}) and procaine penicillin-G (100,000 IU kg^{-1}) were used as analgesic and antibiotic, respectively. Prior to experiments, animals were allowed to recover for 1 week. During recovery, the catheter was protected from biting by an infusion harness apparatus (Harvard Apparatus, Holliston, MA, USA, BS4-61-0238).

2.4. RIA

Blood samples were withdrawn into vacuumed plastic tubes containing 0.6 TIU mL^{-1} aprotinin (Celliance, Billerica, MA, USA, 7108). The tubes were centrifuged at 1600 g, +4 °C for 15 min. Plasma samples were separated and stored at −70 °C for RIA analysis. The RIA method is based on principle of competitive binding and makes use of polyclonal rabbit antibodies against human OXA and goat anti-rabbit antibody to separate bound and free fraction. A commercially available OXA RIA system (Phoenix Pharmaceuticals, Burlingame, CA, USA, RK-003-30) was used to measure plasma concentrations of OXA. The antiserum used at a dilution of 1:2 with equal volume of plasma. Samples were counted with gamma counter and were recorded as count per minute (CPM). OXA concentrations were expressed as pg mL^{-1} according to standard curve. The lowest detectable concentration was 2 pg mL^{-1}.

2.5. Measurement of gastric emptying

The gastric emptying was measured according to the spectrophotometric method of Tache et al. [47]. Briefly, rats received 1.5 mL of test meal contains 50 mg of phenol red (Sigma–Aldrich, St. Louis, MO, USA, P-3532) in 100 mL of 1.5% (w:v) methylcellulose (Sigma–Aldrich, St. Louis, MO, USA, M-0262) solution by oral gavage. Rats were sacrificed by cervical dislocation 30 min after and stomachs were taken out with cardiac and pyloric clamping. In each group, two rats were sacrificed immediately after oral gavage as standard. The stomachs were washed with 0.09% saline and dropped into 100 mL of 0.1 N NaOH (Merck, Darmstadt, Germany, C647762) in falcon tubes and homogenized after cutting into small pieces by scissor. Homogenates were allowed for suspension for 1 h at room temperature. 5 mL of each supernatant was added into tubes contain 0.5 mL of 20% (w:v) trichloroaceticacid (Carlo Erba, Rodano, Italy, 411527) and centrifuged at 2800 rpm for 20 min. After centrifugation, supernatants were added onto 4 mL of 0.5N NaOH and absorbances were measured spectrophotometrically at 560 nm. The gastric emptying (%) was calculated as: 1 − [amount of phenol red obtained from the test stomach

(mg mL^{-1})]/[average amount of phenol red obtained from the standard stomachs (mg mL^{-1})] × 100.

2.6. Motility recording

Gastric MMC was recorded in conscious and freely moving rats according to the manometric-based method of Kihara et al. [25]. Each recording session was started at 10:00 a.m. The catheter was connected to pressure transducer (Hugo Sachs, March-Hugstetten, Germany, P75-Type 379) with a dual channel infusion swivel (Harvard Apparatus, Holliston, MA, USA, BS4-61-0003) to allow free movement. Stomach was continuously infused with 0.9% saline at a rate of 2 mL h^{-1} by an infusion pump (Kent Scientific Corporation, Torrington, CT, USA, Genie Plus). Data were recorded with a data acquisition system (Biopac Systems, Goleta, CA, USA, MP-150) and stored in a portable computer. Data analysis was performed with AcqKnowledge version 3.7.3 software. Clustered pressure increases above 10 cm H_2O were defined as phase III-like contractions. Motility index, peak amplitude, duration of MMC, phase I and phase III were used for comparison of experimental groups. Motility index was calculated as area under the curve; whereas, the duration of MMC was accepted as the interval between onsets of consecutive phase III-like contractions. Motility index and peak amplitude changes were calculated from the two phases and expressed as the percentage of phase III/phase I.

2.7. Western blot

The stomach and hypothalamus were stored in liquid nitrogen for determination of PPO expression. On experiment day, the tissues were taken out from liquid nitrogen and cut into small pieces on ice. 600 μL lysis buffer containing 1% SDS (Sigma–Aldrich, St. Louis, MO, USA, L-4509), 1 mM sodium orthovanadate (Sigma–Aldrich, St. Louis, MO, USA, S6508), 10 mM Tris pH 7.4 (Sigma–Aldrich, St. Louis, MO, USA, T1503) was added into eppendorf tubes for 0.2 g tissue. In addition, 50 μL protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO, USA, P2714) was added for 1000 μL of tissue-buffer mixture. The mixture incubated on ice for 30 min then sonicated for 30 s (Bandelin Electronic, Berlin, Germany, UW2070). After sonication, samples were centrifuged at 15,000 rpm at 4 °C and supernatants were solubilized in Laemmli sample buffer (Sigma–Aldrich, St. Louis, MO, USA, S3401). Prior to electrophoresis, samples were boiled for 5 min at 95 °C. Amounts of protein were determined with a protein assay kit (Pierce, Rockford, IL, USA, R3236) according to Bradford [4] and 50 μg of total protein was loaded on SDS-polyacrylamide gel with accompaniment of molecular weight marker (Amersham Biosciences, Buckinghamshire, UK, RPN 755). Commercial rat OXA was used as positive control (Sigma–Aldrich, St. Louis, MO, USA, O-6012). The gel was run at 100 V, 50 mA on 18% gradient gel, followed by transfer onto nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA, USA, 162-0112) at 4 °C and subsequently hybridized with the primary antibody (Chemicon, Temecula, CA, USA, AB-3098) and β-actin (Abcam, Cambridge, MA, USA, ab8227). After incubation with the primary antibody at +4 °C for 24 h, membranes were washed with TBS-Tween-20 buffer for 1 h and incubated with the horse radish peroxidase (HRP)-conjugated secondary antibody (dilution 1:3000) (Chemicon, Temecula, CA, USA, AP132P) dissolved in TBS-Tween-20 buffer containing 5% non-fat milk (Bio-Rad Laboratories, Hercules, CA, USA, 170-6404) at room temperature for 1 h. Blots were visualized using chemilucent detection system kit according to instructions of manufacturer's protocol (Chemicon, Temecula, CA, USA, 2600). Membranes were exposed to Hyperfilm (Amersham Biosciences, Buckinghamshire, UK, RPN3103K) which was subsequently analyzed using Image J, 1.37v software.

2.8. Immunohistochemistry

Tissue samples were fixed in 10% formaline fixative. After dehydration in ethanol, tissues were cleared in xylene and embedded in paraffin. Subsequently, serial sections (5 μm) were mounted on poly-L-lysine-coated slides (Menzel-Glaser, Braunschweig, Germany, Superfrost). After deparaffinization in xylene, slides were rehydrated through a graded series of ethanol. To unmask antigens, an antigen retrieval procedure was performed by treating the samples in 10 mM citrate buffer, pH 6.0, in a microwave oven at 750 W for 5 min twice. After cooling for 20 min at room temperature, the sections were washed in phosphate buffered saline (PBS; pH 7.4). Endogenous peroxidase activity was blocked by incubation in methanol containing 3% H_2O_2 for 30 min and washed with PBS three times. Afterwards, sections were incubated in a blocking serum (LabVision Corporation, Fremont, CA, USA, TA-125UB) for 7 min at room temperature in order to block non-specific binding. Primer antibody (Chemicon, Temecula, CA, USA, AB-3098) was applied as 1:200 dilution for 1 h at room temperature. Negative control stainings were performed by replacing the primary antibody with the appropriate non-immune IgG. Sections were rinsed three times in PBS and treated with biotinylated secondary antibody and a peroxidase labelled streptavidin (Dako, Carpinteria, CA, USA, K0609), respectively, for 30 min with each of the steps being followed by three rinses in PBS. Peroxidase activity was visualized with AEC (3-amino-9-ethylcarbazol) (LabVision Corporation, Fremont, CA, USA, TA-125-UD) chromogen. Sections were counterstained with hematoxylin, dehydrated, mounted, and examined by light microscopy. Photomicrographs were taken with an Axioplan microscope (Carl Zeiss, Oberkochen, Germany). All samples were exposed to the same protocol and were stained using the same duration of staining. Stained sections were evaluated using an Axioplan microscope (Zeiss, Oberkochen, Germany) with a special ocular scale. From four randomly selected slides, each of three different fields at 40× magnification, were evaluated for the analysis of immunohistochemical staining. The distribution of immunoreactive cells in all experimental groups was also determined semi-quantitatively: 0: negative, (+): weak positive, +: positive, ++: strong positive, +++: very strong positive.

2.9. Drugs

OX1R antagonist SB-334867 (Tocris Biosciences, Ellisville, MO, USA, 1960) was dissolved in dimethyl sulfoxide (Sigma–Aldrich, St. Louis, MO, USA, D8418) and administered immediately before the experimental procedures with dose of 10 mg kg^{-1} in a volume of 100 μL kg^{-1} through the tail venous under light ether anesthesia.

2.10. Data analysis

Data are presented as the means ± SE. Statistical analyses were performed with SPSS version 13.0 software by using Kruskal Wallis and Mann–Whitney *U*-test. The level of significance was accepted as $p < 0.05$.

3. Results

3.1. Time course effect of fasting on plasma OXA

In 0 h-fasted animals, plasma OXA level was 8.89 ± 0.56 pg mL^{-1}. However, there was no significant increase upon 18 h (9.69 ± 0.75 pg mL^{-1}) and 24 h (12.73 ± 1.68 pg mL^{-1}) fasting. The maximal level of OXA was measured in 36 h-fasted animals (27.12 ± 4.22 pg mL^{-1}) which was significantly ($p < 0.01$) higher compared to 0 h fasting. Fasting for 48 h (6.61 ± 0.93 pg mL^{-1}) and 72 h (7.18 ± 1.23 pg mL^{-1}) did not cause any significant change

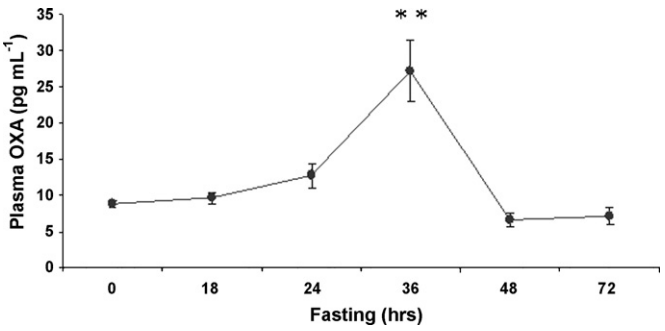


Fig. 1. Time course effect of fasting on plasma OXA concentration (pg mL^{-1}). OXA was assayed in 0, 18, 24, 36, 48 and 72 h-fasted rats ($n = 10$ in each group, $^{**}p < 0.01$ vs 0 h fasting).

compared to 0 h-fasted rats. Thus, 36 h fasting was used to stimulate endogenous OXA synthesis in gastric emptying and motility experiments (Fig. 1).

3.2. Effect of 36 h fasting on gastric emptying

In 18 h fasting group, gastric emptying was significantly ($p < 0.01$) higher than Fed group. Likewise, 36 h fasting significantly ($p < 0.001$) increased the gastric emptying compared to 18 h fasting group. In 36 h fasting-SB group, SB-334867 completely abolished the effect of 36 h fasting and significantly ($p < 0.001$) decreased the gastric emptying; whereas, vagotomy did not alter. However, vagotomy significantly ($p < 0.05$) augmented the effect of SB-334867. In 18 h fasting-SB and 18 h fasting-VG groups, gastric emptying was significantly ($p < 0.01$) decreased compared to 18 h fasting group (Fig. 2).

3.3. Effect of 36 h fasting on interdigestive motility

Compared to 18 h fasting, 36 h fasting caused a significant ($p < 0.001$) increase in MMC duration (Fig. 3A). This effect was completely ($p < 0.001$) abolished with SB-334867 while vagotomy did not cause any change. In 36 h fasting-VG-SB group, SB-334867 administration inhibited the effect of 36 h fasting comparable to 36 h fasting-SB group. In 18 h-fasted animals, vagotomy significantly ($p < 0.01$) increased the MMC duration while SB-334867 significantly ($p < 0.001$) decreased compared to control (Fig. 4A). The duration of phase III was not changed with any of the treatments (Figs. 3B and 4B).

36 h fasting significantly ($p < 0.01$) decreased the peak amplitude and motility index changes as well as MMC frequency compared to 18 h fasting. SB-334867 completely restored the inhibitory effects of 36 h fasting; ($p < 0.001$ in peak amplitude change and MMC frequency; $p < 0.01$ in motility index change) whereas, vagotomy did not change. In 36 h fasting-VG-SB group, SB-334867 administration restored the effect of 36 h fasting comparable to the 36 h fasting-SB group. In 18 h-fasted animals, SB-334867 administration significantly increased the peak amplitude change ($p < 0.01$), motility index change ($p < 0.01$) and MMC frequency ($p < 0.001$), while vagotomy treatment significantly ($p < 0.01$) decreased (Table 1).

3.4. Effect of 36 h fasting on plasma OXA concentration

18 h fasting did not lead to any change in plasma OXA compared to the Fed group. In 36 h-fasted animals, plasma OXA was significantly ($p < 0.001$) higher compared to that of their controls. Among the 36 h fasting groups, SB-334867 caused a significant ($p < 0.05$) decrease, while vagotomy caused a slight attenuation in plasma OXA concentration. However, the maximal ($p < 0.01$) decrease was observed in 36 h fasting-VG-SB group, compared to the other 36 h-fasted groups (Fig. 5).

3.5. Effect of 36 h fasting on preproorexin expression

The blotting results of hypothalamus and stomach are presented in Fig. 6A and B, respectively. PPO expression was significantly ($p < 0.05$) increased in both hypothalamus and stomach upon 18 and 36 h of fasting. However, the highest expression was observed in 36 h-fasted animals. With 18 h fasting, vagotomy or SB-334867 alone did not change the PPO expression (Fig. 6C).

3.6. Effect of 36 h fasting on OXA immunoreactivity

In immunohistochemical studies, OXA immunoreactivity was increased upon 18 h and 36 h fasting compared to Fed group (Fig. 7). The maximal immunoreactivity was observed in 36 h fasting groups (36 h fasting, 36 h fasting-SB, 36 h fasting-VG, 36 h fasting-VG-SB). Vagotomy or SB-334867 did not affect the OXA immunoreactivity (Fig. 8). The distribution and intensity of OXA-immunopositive cells are presented in Table 2.

4. Discussion

Current data demonstrates the peripheral effects of endogenous OXA on gastric motility. Long-term fasting induced the endogenous OXA, which facilitated gastric emptying and inhibited interdigestive gastric motility. OXA increased the duration of MMC; whereas, decreased maximal amplitude and motility index changes. These effects seemed like N.vagus-independent. The gastrokinetic effects of OXA were examined with selective OX1R antagonist SB334867

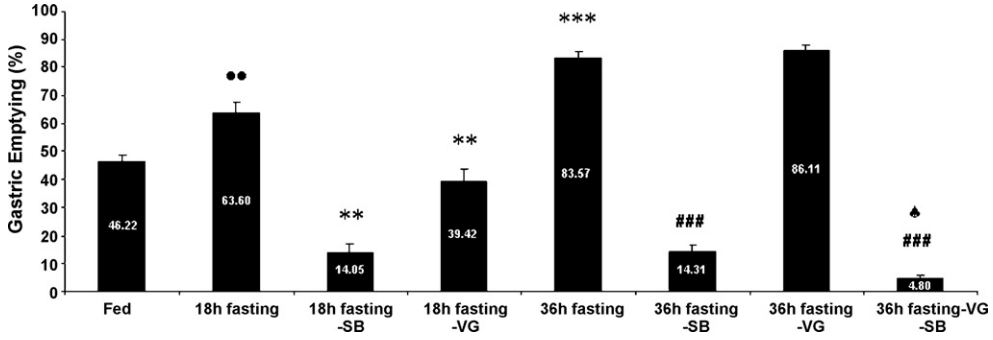


Fig. 2. Effect of fasting-induced endogenous OXA on gastric emptying. Fed, no fasting; 18 h fasting, underwent fasting for 18 h; 18 h fasting-SB, underwent fasting for 18 h and i.v. administration of SB-334867; 18 h fasting-VG, underwent fasting for 18 h and vagotomy treatment; 36 h fasting, underwent fasting for 36 h; 36 h fasting-SB, underwent fasting for 36 h and i.v. administration of SB-334867; 36 h fasting-VG, underwent fasting for 36 h and vagotomy treatment; 36 h fasting-VG-SB, underwent fasting for 36 h and vagotomy treatment, and i.v. administration of SB-334867 ($n = 9$ in each group, $^{\bullet\bullet}p < 0.01$ vs Fed, $^{**}p < 0.01$ vs 18 h fasting, $^{***}p < 0.001$ vs 18 h fasting, $^{\#\#\#}p < 0.001$ vs 36 h fasting, $^{\spadesuit}p < 0.05$ vs 36 h fasting-SB).

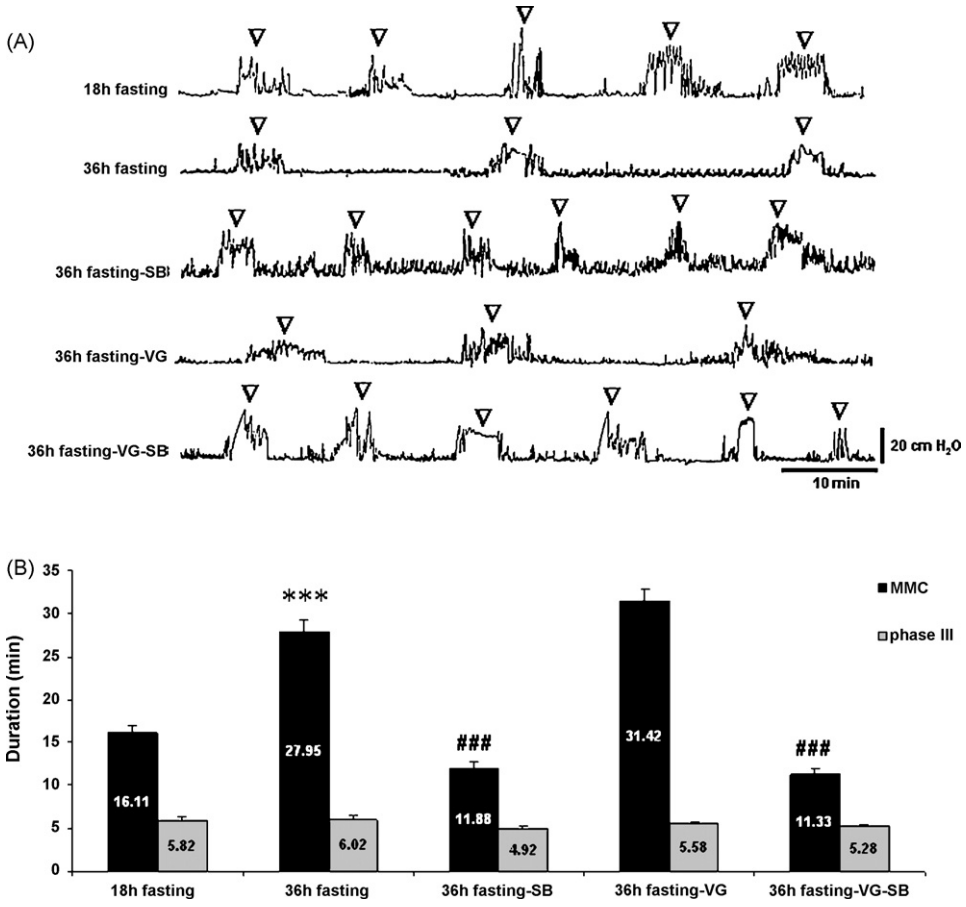


Fig. 3. Gastric motor activities in conscious rats. In fasting state, regular spontaneous phase III-like contractions were recorded from antrum (A). 36 h fasting increased the interval of phase III-like contractions without affecting the duration (B). (▽) The phase III-like contractions. 18 h fasting, underwent fasting for 18 h; 36 h fasting, underwent fasting for 36 h; 36 h fasting-SB, underwent fasting for 36 h and i.v. administration of SB-334867; 36 h fasting-VG, underwent fasting for 36 h and vagotomy treatment; 36 h fasting-VG-SB, underwent fasting for 36 h and vagotomy treatment and i.v. administration of SB-334867 ($n = 8$ in each group, $^{***}p < 0.001$ vs 18 h fasting, $^{\#\#\#}p < 0.001$ vs 36 h fasting).

and found OX1R-mediated. 36 h fasting significantly increased plasma OXA concentration, PPO expression and OXA immunoreactivity in stomach. In 36 h-fasted rats, SB-334867 induced an attenuation in plasma OXA concentration which was potentiated by vagotomy. In contrast, PPO expression and OXA immunoreactivity were not altered with SB-334867.

Upon fasting, increased levels of plasma OXA was previously shown in rat [40] and human [29]. In our preliminary study, maximal plasma OXA was measured with 36 h fasting. In contrast, in 48 h and 72 h-fasted animals, OXA concentrations were measured slightly <0 h-fasted group. The mechanism of this reduction is not fully understood. There are contradictive findings about fasting and OXA. Komaki showed significant increase in plasma OXA in 72 h-fasted individuals [29]. In contrast, decreased hypothalamic PPO expression [24] and neuronal activity of OXA-producing cells [41] was showed in rats upon 72 and 84 h fasting, respectively. The discrepancy of data may be attributed to distinct endocrine and metabolic responses of rodent and human.

Fasting is frequently used in energy homeostasis studies. In most species, fasting consist of three phases. The first phase, defined as an adaptive phase, which takes place in first 24 h, involves over-expression of hypothalamic peptides. The second phase starts

Table 1
Effect of 36 h fasting on phase I and phase III-like contractions of gastric MMC.

	Peak amplitude change (%) (phase III/phase I)	Motility index change (%) (phase III/phase I)	MMC frequency (cycle, h^{-1})
18 h fasting	266.38 ± 12.77	225.01 ± 10.11	3.7 ± 0.43
18 h fasting-SB	344.25 ± 21.32**	304.17 ± 16.11**	5.6 ± 0.31***
18 h fasting-VG	182.38 ± 17.59**	125.33 ± 5.69**	2.9 ± 0.26**
36 h fasting	193.88 ± 12.55**	148.03 ± 11.63**	2.1 ± 0.13***
36 h fasting-SB	318.38 ± 27.60###	244.20 ± 19.19##	5.0 ± 0.14###
36 h fasting-VG	164.14 ± 15.83	123.07 ± 9.67	1.9 ± 0.12
36 h fasting-VG-SB	305.13 ± 12.13###	241.95 ± 18.15##	5.3 ± 0.21###

18 h fasting, underwent fasting for 18 h; 18 h fasting-SB, underwent fasting for 18 h and i.v. administration of SB-334867; 18 h fasting-VG, underwent fasting for 18 h and vagotomy treatment; 36 h fasting, underwent fasting for 36 h; 36 h fasting-SB, underwent fasting for 36 h and i.v. administration of SB-334867; 36 h fasting-VG, underwent fasting for 36 h and vagotomy treatment; 36 h fasting-VG-SB, underwent fasting for 36 h, vagotomy treatment and i.v. administration of SB-334867.

** $p < 0.01$ vs 18 h fasting, $n = 5$ in each group.

*** $p < 0.001$ vs 18 h fasting, $n = 5$ in each group.

$p < 0.01$ vs 36 h fasting, $n = 5$ in each group.

$p < 0.001$ vs 36 h fasting, $n = 5$ in each group.

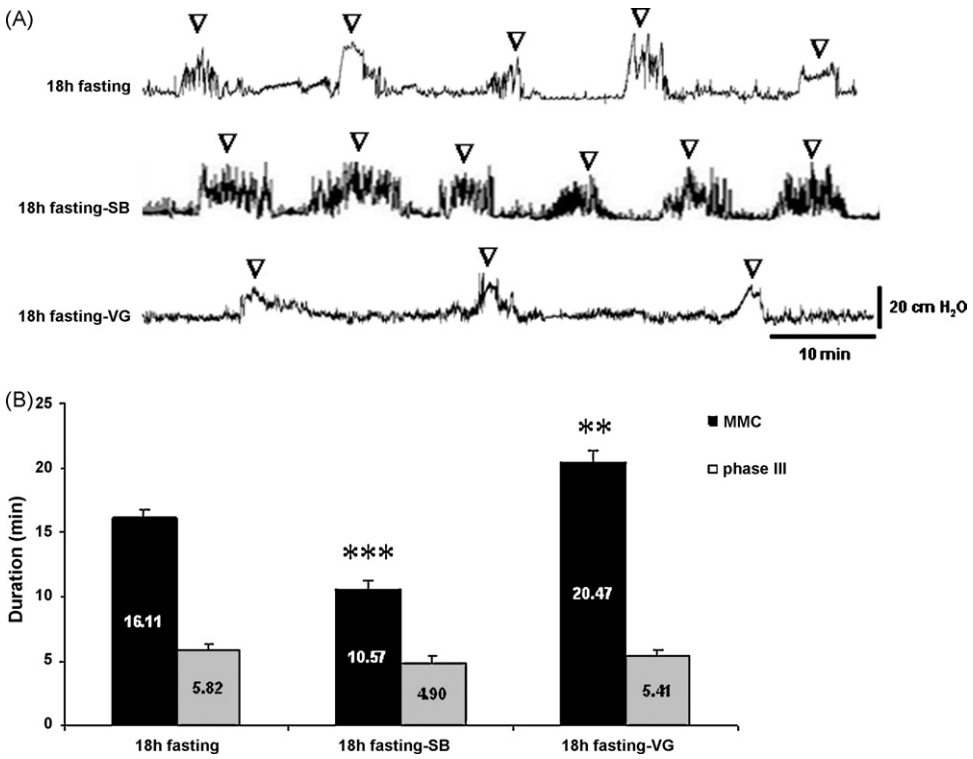


Fig. 4. Gastric motor activities in 18 h-fasted conscious rats. Regular spontaneous phase III-like contractions were recorded from antrum (A). SB-334867 shortened the intervals of phase III-like contractions; whereas, vagotomy prolonged without affecting the duration (B). (▽) The phase III-like contractions. 18 h fasting, underwent fasting for 18 h; 18 h fasting-SB, underwent fasting for 18 h and i.v. administration of SB-334867; 18 h fasting-VG, underwent fasting for 18 h and vagotomy treatment ($n = 8$ in each group, $^{**}p < 0.01$ vs 18 h fasting, $^{***}p < 0.001$ vs 18 h fasting).

in 2–4 day of fasting: the organism starts to maintain energy from oxidation of lipids due to lack of glucose. In third phase, proteins are used as energy source [24]. In prolonged fasting conditions, CNS decreases glucose transport capacity while increasing uptake of ketone bodies as an adaptive mechanism [20]. Lateral hypothalamus, where the orexinergic neurons have dense situation, implicated in control of feeding and body weight. The glucose-sensitive neurons within lateral hypothalamic area have been identified that responding to feeding-related signals including changes in glucose and presence of food in gut. However, some of these neurons are inhibited by ketones [6]. We suggest that decreases in plasma OXA, after 36 h fasting may be involved with inhibition of hypothalamic OXA signaling. In our results, SB-334867 had an inhibitor effect on plasma OXA levels, which was augmented with vagotomy. The mechanism of SB-334867 induced down-regulation is not clear. However, interruption of vagal signaling potentiated this mechanism.

The proximal and distal stomach has distinct motor functions. Proximal part serves as storage with its high compliance capacity; whereas, the distal part acts as a grinder, chops and grinds meal into chime by its powerful contractile activity [9,17]. Gastric emptying is determined by simultaneous contractions of antrum and pylorus [5]. However, the stomach parts that directly affected by OXA is not known. Baccari and Calamai showed the OXA-induced contractions which were abolished with L-NNA or SB-334867 administrations in mouse fundus strips. On the other hand, OXA had no effect on neither spontaneous nor electrical stimulation-induced contractions in antral strips [3]. Since OXA has hyperphagic effects, OXA-dependent relaxation of proximal stomach may be explained as an adaptive response for over-nutrition.

It remains unclear whether endogenous OXA is directly involved in gastric emptying. Ehrstrom previously demonstrated inhibitory effect of OXA on small intestine MMC in rats which was abolished by nitric oxide synthase (NOS) inhibitor L-NNA [15]. These results

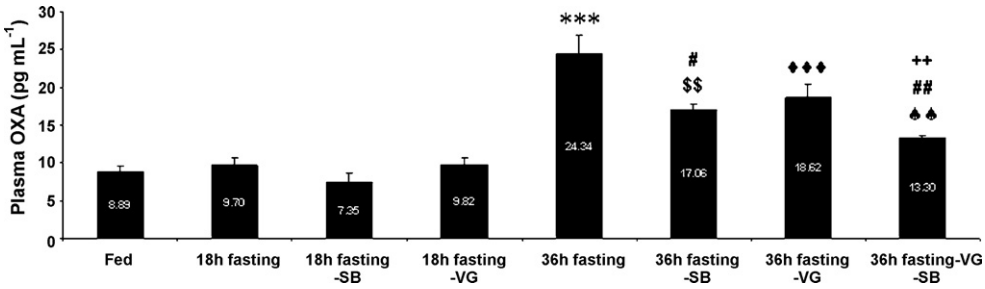


Fig. 5. Effect of 36 h fasting on plasma OXA concentration. OXA levels were increased upon fasting. Fed, no fasting; 18 h fasting, underwent fasting for 18 h; 18 h fasting-SB, underwent fasting for 18 h and i.v. administration of SB-334867; 18 h fasting-VG, underwent fasting for 18 h and vagotomy treatment; 36 h fasting, underwent fasting for 36 h; 36 h fasting-SB, underwent fasting asted for 36 h and i.v. administration of SB-334867; 36 h fasting-VG, underwent fasting for 36 h and vagotomy treatment; 36 h fasting-VG-SB, underwent fasting for 36 h, vagotomy treatment and i.v. administration of SB-334867 ($n = 8$ in each group, $^{***}p < 0.001$ vs 18 h fasting, $^{\#}p < 0.05$ vs 36 h fasting, $^{\#\#}p < 0.05$ vs 36 h fasting, $^{\$\$}p < 0.01$ vs 18 h fasting-SB, $^{\blacklozenge\blacklozenge\blacklozenge}p < 0.001$ vs 18 h fasting-VG, $^{\spadesuit\spadesuit}p < 0.01$ vs 36 h fasting-SB, $^{++}p < 0.01$ vs 36 h fasting-VG.

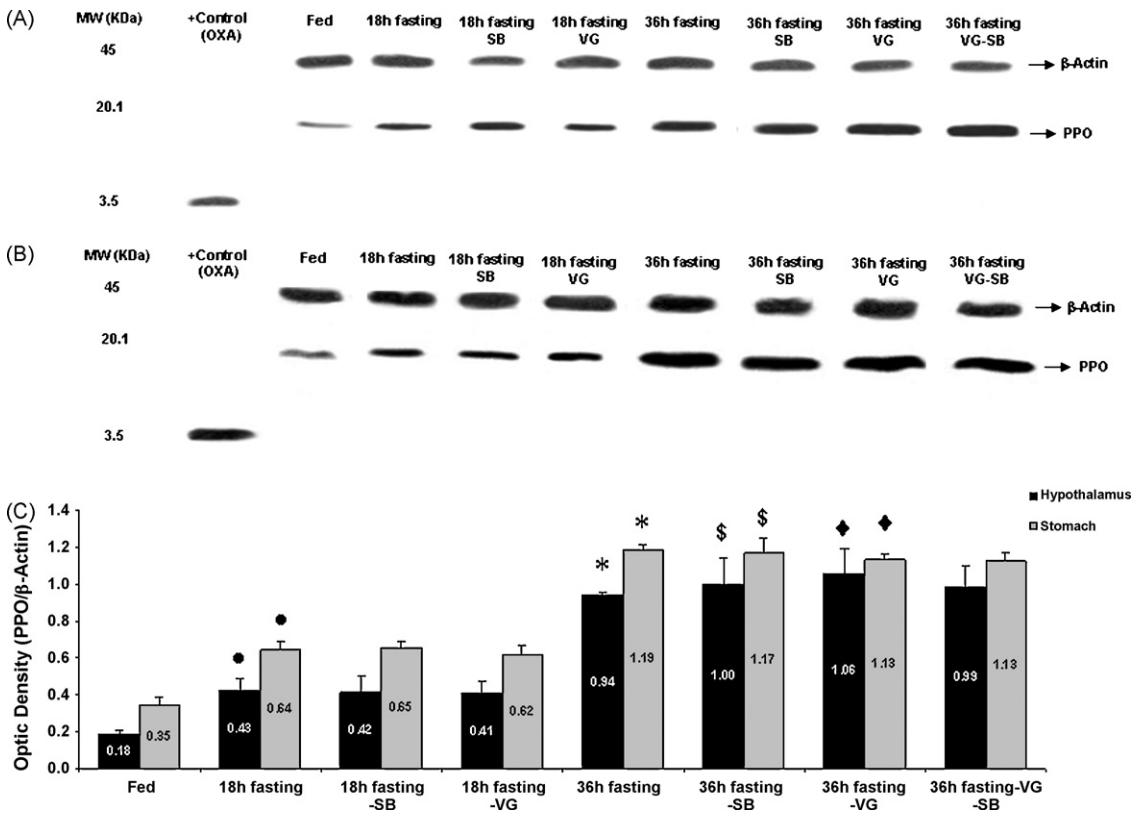


Fig. 6. Effect of 36 h fasting on PPO expression. In hypothalamus (A) and stomach (B), the immunoreactive bands, corresponding to PPO and β-actin were obtained nearby 20.1 and 45 kDa molecular markers, respectively. Commercial rat OXA was used as positive control. PPO expression was increased upon fasting in a time-dependent manner (C). Fed, no fasting; 18 h fasting, underwent fasting for 18 h; 18 h fasting-SB, underwent fasting for 18 h and i.v. administration of SB-334867; 18 h fasting-VG, underwent fasting for 18 h and vagotomy treatment; 36 h fasting, underwent fasting for 36 h; 36 h fasting-SB, underwent fasting for 36 h and i.v. administration of SB-334867; 36 h fasting-VG, underwent fasting for 36 h and vagotomy treatment; 36 h fasting-VG-SB, underwent fasting for 36 h, vagotomy treatment and i.v. administration of SB-334867. PPO, preproorexin and MW (kDa), molecular weight in kilodalton ($n = 4$ in each group, $^{\bullet}p < 0.05$ vs Fed, $^{*}p < 0.05$ vs 18 h fasting, $^{\$}p < 0.05$ vs 18 h fasting-SB, $^{\blacklozenge}p < 0.05$ vs 18 h fasting-VG).

suggest that inhibitory effect of OXA might be mediated by nitrergic pathway. Ehrstrom also showed a group of OXA-immunoreactive cells, co-localized with nNOS in the myenteric plexus. Nitric oxide (NO) is a well-known regulator of gastric emptying [52]. Inhibition of NOS decreases gastric emptying in rats and humans [8,52]. In rats, NO facilitates gastroduodenal transit via reducing pyloric and duodenal tonus [49]. Therefore, stimulatory effect of OXA on gastric emptying may be dependent on NO-dependent inhibition of pyloric or duodenal tonus.

We intravenously administered selective OX1R antagonist, SB-334867 with dose of 10 mg kg^{-1}, to test OX1R-mediated pathway. Efficiency of this dose was previously studied in rats [14,15]. As it was completely abolished with SB-334867, effect OXA on gastric emptying seems like OX1R-mediated. SB-334867 not only abolished the increased gastric emptying, but also reduced the emptying rate under the Fed group. This reduction may be attributed to the inhibition of basal endogenous OXA.

We performed subdiaphragmatic vagotomy for blocking N.vagus-mediated effects of OXA. The surgical achievement was verified with gastric acid measurement. In vagotomized rats, gastric acid output was significantly lower than their intact littermates. It was previously shown that vagotomy decreased the gastric emptying in rats [36]. We established a significant reduction in gastric emptying 18 h fasting-VG group. However, vagotomy did not affect gastric emptying in 36 h fasting-VG group. Likewise, Ehrstrom concluded that vagotomy did not change effect of exogenous OXA on small intestine MMC in rats [15]. According to our data, in prolonged fasting conditions, endogenous OXA acts by a paracrine mechanism on gastric emptying rather than CNS-mediated action. In 36 h fasting-VG-SB group, gastric emptying was significantly decreased compared to 36 h fasting-SB group. There might be an unknown interaction between OX1R and N.vagus during long-term fasting. The mode of action could not be explained with well-known mechanisms of vagal signaling. Further investigations are needed to clarify this result.

Table 2
The semi-quantitative distribution of OXA-immunoreactive cells and immunostaining intensities.

	Epithelial cells	Glandular cells	Mucosal endocrine cells	Myenteric plexus neurons
Fed	0	(+)	++	(+)
18 h fasting	+	++	++	+
18 h fasting-SB	(+)	++	++	++
18 h fasting-VG	(+)	+	++	++
36 h fasting	(+)	++	+++	+++
36 h fasting-SB	+	++	++	+++
36 h fasting-VG	(+)	+	+++	+++
36 h fasting-VG-SB	(+)	++	++	+++

Fed, no fasting; 18 h fasting, underwent fasting for 18 h; 18 h fasting-SB, underwent fasting for 18 h and i.v. administration of SB-334867; 18 h fasting-VG, underwent fasting for 18 h and vagotomy treatment; 36 h fasting, underwent fasting for 36 h; 36 h fasting-SB, underwent fasting for 36 h and i.v. administration of SB-334867; 36 h fasting-VG, underwent fasting for 36 h and vagotomy treatment; 36 h fasting-VG-SB, underwent fasting for 36 h, vagotomy treatment and i.v. administration of SB-334867. 0: negative; (+): weak positive; +: positive; ++: dense positive; +++: very dense positive.

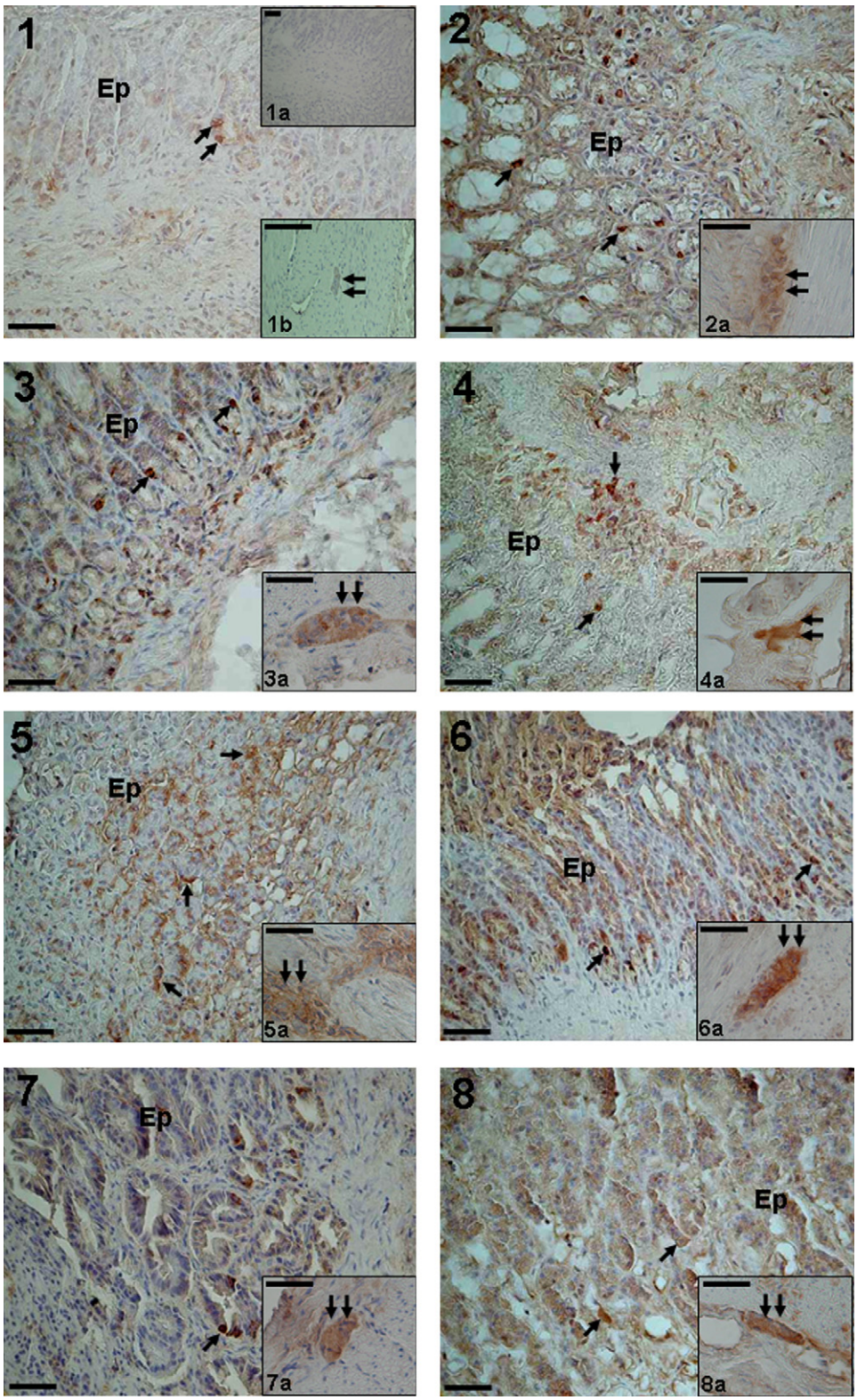


Fig. 7. OXA immunoreactivity in stomach. 1: Fed (no fasting), magnification = 40×, scale bar = 75 μm; 1a: negative control, magnification = 40×, scale bar = 75 μm. 1b: myenteric plexus, magnification = 20×, scale bar = 150 μm. 2: 18 h fasting (underwent fasting for 18 h), magnification = 40×, scale bar = 75 μm; 2a: myenteric plexus, magnification = 40×, scale bar = 75 μm. 3: 18 h fasting-SB (underwent fasting for 18 h and i.v. administration of SB-334867), magnification = 40×, scale bar = 75 μm; 3a: myenteric plexus, magnification = 40×, scale bar = 75 μm. 4: 18 h fasting-VG (underwent fasting for 18 h and vagotomy treatment), magnification = 40×, scale bar = 75 μm; 4a: myenteric plexus, magnification = 40×, scale bar = 75 μm. 5: 36 h fasting (underwent fasting for 36 h), magnification = 40×, scale bar = 75 μm; 5a: myenteric plexus, magnification = 40×, scale bar = 75 μm. 6: 36 h fasting-SB (underwent fasting for 36 h and i.v. administration of SB-334867), magnification = 40×, scale bar = 75 μm; 6a: myenteric plexus, magnification = 40×, scale bar = 75 μm. 7: 36 h fasting-VG (underwent fasting for 36 h and vagotomy treatment), magnification = 40×, scale bar = 75 μm; 7a: myenteric plexus, magnification = 40×, scale bar = 75 μm. 8: 36 h fasting-VG-SB (underwent fasting for 36 h, vagotomy treatment and i.v. administration of SB-334867), magnification = 40×, scale bar = 75 μm; 8a: myenteric plexus, magnification = 40×, scale bar = 75 μm. Single arrow: mucosal endocrine cells, double arrow: myenteric plexus neurons, Ep: epithelial cells.

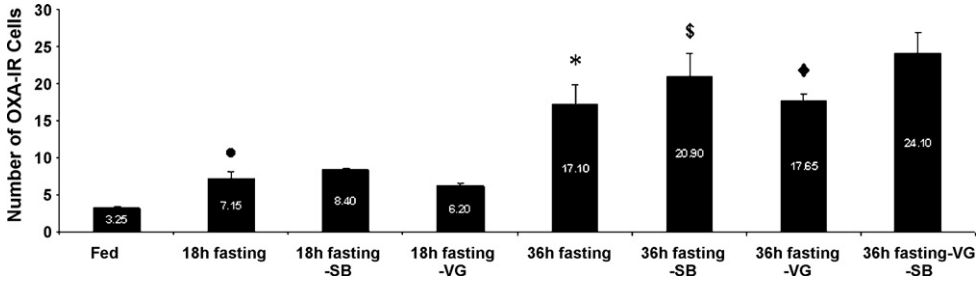


Fig. 8. Number of OXA-immunoreactive cells in stomach. OXA immunoreactivity was established within the antrum region of stomach. The quantity of positive cells was increased in response to fasting. Fed, no fasting; 18 h fasting, underwent fasting for 18 h; 18 h fasting-SB, underwent fasting for 18 h and i.v. administration of SB-334867; 18 h fasting-VG, underwent fasting for 18 h and vagotomy treatment; 36 h fasting, underwent fasting for 36 h; 36 h fasting-SB, underwent fasting for 36 h and i.v. administration of SB-334867; 36 h fasting-VG, fasted for 36 h and vagotomy treatment; 36 h fasting-VG-SB, underwent fasting for 36 h, vagotomy treatment and i.v. administration of SB-334867 ($n = 5$ in each group, $^{\bullet}p < 0.05$ vs Fed, $^{*}p < 0.05$ vs 18 h fasting, $^{\$}p < 0.05$ vs 18 h fasting-SB, $^{\blacklozenge}p < 0.05$ vs 18 h fasting-VG).

During interdigestive state, gastrointestinal motility is characterized by a coordinated motor pattern, called MMC [1,7]. MMC consists of three phases: phase I (period of motor quiescence), phase II (period of irregular low-amplitude contractions), and phase III (period of regular high-amplitude contractions). In humans and dogs, MMC is observed every 90–120 min. In contrast, in rats, MMC cycle is <20 min and not as regular as humans and dogs [1]. Myenteric plexus plays the main role for initiation and migration of MMC [19,51]. N.vagus is involved in postprandial disruption rather than initiation. However, vagotomy inhibits phase III-like contractions while prolonging MMC cycle duration [19,51]. In concordance with previous studies, MMC duration was measured approximately 16 min in control animals [1,2,7,15,37,50]. 36 h fasting increased the MMC duration without altering phase I; whereas, decreased the maximal amplitude and motility index changes (% of phase III/phase I). SB-334867 administration abolished these effects. Therefore it can be suggested that the role of OX1R-mediated signaling in OXA-dependent regulation of MMC.

It has been reported that NO/cGMP transduction mechanism is involved in the mechanism of MMC. Sodium nitroprusside-induced MMC inhibition and L-NNA-induced phase III-like contractions have been shown in rats [42]. Ehrstrom previously showed that MCC inhibition by exogenous OXA was partly dependent on activation of the L-arginine/NO pathway [15]. Besides NO, vasoactive intestinal peptide (VIP) has an inhibitor effect on MMC activity. VIP induced inhibition may occur either direct or NO-mediated manner [22,33,45]. Naslund et al. previously established that myenteric and submucosal OXA immunoreactive neurons also contain VIP in rats [37]. The inhibitory effect of OXA on MMC might be mediated by VIP and/or NO.

In parallel to gastric emptying, we demonstrated that in all SB-334867-treated groups, MMC duration was decreased under control levels, while motility index and peak amplitude changes were calculated over control values. The possible reason of these similar results may be explained with inhibition of endogenous OXA. On the other hand, subdiaphragmatic vagotomy had a significant ($p < 0.01$) inhibitory effect on MMC which was comparable to 36 h fasting treatment. Interestingly, vagotomy was only effective in 18 h fasting conditions. There was no significant difference between 36 h fasting and 36 h fasting-VG groups. Similar to our data, Ehstrom, established a twofold increase in MMC duration by subdiaphragmatic vagotomy, which was ineffective on OXA-induced inhibition [15]. We established the increased PPO expression and OXA immunoreactivity in stomach with fasting. Therefore, we suggest that increased OXA activity in myenteric plexus may act on MMC via neural and/or paracrine mechanisms.

PPO is the common precursor molecule of OXA and OXB [27,44]. In Western blot studies we evaluated PPO as an indicator of OXA expression. We found increased PPO expressions in 18 h-fasted groups compared with Fed group [34,38,41,43,53,54]. However, the highest expressions were obtained in 36 h-fasted groups. In contrast to plasma OXA, SB-334867 did not affect the PPO expressions in 36 h-fasted rats. Although, the mechanism is still unclear, it may be speculated that SB-334867 has an inhibitor effect on proteolytic cleavage involved in OXA synthesis.

The OXA immunoreactivity has been shown within alimentary tract of rat [15,37] and human [13]. In immunohistochemical studies, we observed immunoreactive cells in antrum but not in fundus or corpus. Concordant with previous reports, we found increased OXA immunoreactivity upon fasting. The strongest immunoreactions were established in myenteric plexus and mucosal endocrine cells of 36 h-fasted rats [13,14,26,27,37].

5. Conclusions

OXA is stimulated in response to 36 h fasting in peripheral and CNS tissues. Endogenous OXA facilitates gastric emptying while inhibits MMC via OX1R-mediated, N.vagus-independent pathway.

Acknowledgements

This study was supported by the Research Foundation of Akdeniz University (Project number: 2005.03.0122.007). The authors thank Erol Nizamoğlu for technical assistance in surgical operations and Sibel Özer for generous effort in immunohistochemical assay.

References

[1] Ariga H, Imai K, Chen C, Mantyh C, Pappas TN, Takahashi T. Fixed feeding potentiates interdigestive gastric motor activity in rats: importance of eating habits for maintaining interdigestive MMC. American Journal of Physiology – Gastrointestinal and Liver Physiology 2008;294:G655–9.
[2] Ariga H, Tsukamoto K, Chen C, Mantyh C, Pappas TN, Takahashi T. Endogenous acyl ghrelin is involved in mediating spontaneous phase III-like contractions of the rat stomach. Neurogastroenterology and Motility 2007;19:675–80.
[3] Baccari MC, Calamai F. Influence of orexin A on the mechanical activity of mouse gastric strips. Regulatory Peptides 2008;146:67–72.
[4] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Analytical Biochemistry 1976;72:248–54.
[5] Burks TF, Galligan JJ, Porreca F, Barber WD. Regulation of gastric-emptying. Federation Proceedings 1985;44:2897–901.
[6] Cai XJ, Widdowson PS, Harrold J, Wilson S, Buckingham RE, Arch JRS, et al. Hypothalamic orexin expression – modulation by blood glucose and feeding. Diabetes 1999;48:2132–7.
[7] Chung SA, Rotstein O, Greenberg GR, Diamant NE. Mechanisms coordinating gastric and small-intestinal mmc – role of extrinsic innervation rather than motilin. American Journal of Physiology – Gastrointestinal and Liver Physiology 1994;30:G800–9.
[8] Corak A, Coskun T, Alican I, Kurtel H, Yegen BC. The effect of nitric oxide synthase blockade and indomethacin on gastric emptying and gastric contractility. Pharmacology 1997;54:298–304.

[9] Costa M. All together now: from pacemakers to gastric peristalsis. Journal of Physiology – London 2006;571:1.
[10] Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, et al. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. Proceedings of the National Academy of Sciences of the United States of America 1999;96:748–53.
[11] de Lecea L, Sutcliffe JG. The hypocretins/orexins: novel hypothalamic neuropeptides involved in different physiological systems. Cellular and Molecular Life Sciences 1999;56:473–80.
[12] Dube MG, Horvath TL, Kalra PS, Kalra SP. Evidence of NPY Y5 receptor involvement in food intake elicited by orexin A in sated rats. Peptides 2000;21:1557–60.
[13] Ehrstrom M, Gustafsson T, Finn A, Kirchgessner A, Gryback P, Jacobsson H, et al. Inhibitory effect of exogenous orexin a on gastric emptying, plasma leptin, and the distribution of orexin and orexin receptors in the gut and pancreas in man. Journal of Clinical Endocrinology & Metabolism 2005;90:2370–7.
[14] Ehrstrom M, Levin F, Kirchgessner AL, Schmidt PT, Hilsted LM, Gryback P, et al. Stimulatory effect of endogenous orexin A on gastric emptying and acid secretion independent of gastrin. Regulatory Peptides 2005;132:9–16.
[15] Ehrstrom M, Naslund E, Ma J, Kirchgessner AL, Hellstrom PM. Physiological regulation and NO-dependent inhibition of migrating myoelectric complex in the rat small bowel by OXA. American Journal of Physiology – Gastrointestinal and Liver Physiology 2003;285:G688–95.
[16] Fujino K, Inui A, Asakawa A, Kihara N, Fujimura M, Fujimiya M. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. The Journal of Physiology 2003;550:227–40.
[17] Furudono Y, Ando C, Yamamoto C, Kobashi M, Yamamoto T. Involvement of specific orexigenic neuropeptides in sweetener-induced overconsumption in rats. Behavioural Brain Research 2006;175:241–8.
[18] Grabauskas G, Moises HC. Gastrointestinal-projecting neurones in the dorsal motor nucleus of the vagus exhibit direct and viscerotopically organized sensitivity to orexin. The Journal of Physiology 2003;549:37–56.
[19] Hansen MB. Small intestinal manometry. Physiological Research 2002;51:541–56.
[20] Hasselbalch SG, Knudsen GM, Jakobsen J, Hageman LP, Holm S, Paulson OB. Blood-brain barrier permeability of glucose and ketone bodies during short-term starvation in humans. American Journal of Physiology 1995;268:E1161–6.
[21] Heinonen MV, Purhonen AK, Makela KA, Herzig KH. Functions of orexins in peripheral tissues. Acta Physiologica (Oxford) 2008;192:471–85.
[22] Hellstrom PM, Ljung T. Nitrergic inhibition of migrating myoelectric complex in the rat is mediated by vasoactive intestinal peptide. Neurogastroenterology and Motility 1996;8:299–306.
[23] Johren O, Neidert SJ, Kummer M, Dendorfer A, Dominiak P. Prepro-orexin and orexin receptor mRNAs are differentially expressed in peripheral tissues of male and female rats. Endocrinology 2001;142:3324–31.
[24] Kappeler L, Zizzari P, Grouselle D, Epelbaum J, Bluet-Pajot MT. Plasma and hypothalamic peptide-hormone levels regulating somatotroph function and energy balance in fed and fasted states: a comparative study in four strains of rats. Journal of Neuroendocrinology 2004;16:980–8.
[25] Kihara N, Fujimura M, Yamamoto I, Itoh E, Inui A, Fujimiya M. Effects of central and peripheral urocortin on fed and fasted gastroduodenal motor activity in conscious rats. American Journal of Physiology – Gastrointestinal and Liver Physiology 2001;280:G406–19.
[26] Kirchgessner AL. Orexins in the brain–gut axis. Endocrine Reviews 2002;23:1–15.
[27] Kirchgessner AL, Liu M. Orexin synthesis and response in the gut. Neuron 1999;24:941–51.
[28] Kobashi M, Furudono Y, Matsuo R, Yamamoto T. Central orexin facilitates gastric relaxation and contractility in rats. Neuroscience Letters 2002;332:171–4.
[29] Komaki G, Matsumoto Y, Nishikata H, Kawai K, Nozaki T, Takii M, et al. Orexin-A and leptin change inversely in fasting non-obese subjects. European Journal of Endocrinology 2001;144:645–51.
[30] Korczynski W, Ceregrzyn M, Matyjek R, Kato I, Kuwahara A, Wolinski J, et al. Central and local (enteric) action of orexins. Journal of Physiology and Pharmacology 2006;57(Suppl. 6):17–42.
[31] Krowicki ZK, Burmeister MA, Berthoud HR, Scullion RT, Fuchs K, Hornby PJ. Orexins in rat dorsal motor nucleus of the vagus potently stimulate gastric motor function. American Journal of Physiology – Gastrointestinal and Liver Physiology 2002;283:G465–72.
[32] Kukkonen JP, Holmqvist T, Ammoun S, Akerman KE. Functions of the orexinergic/hypocretinergic system. American Journal of Physiology – Cell Physiology 2002;283:C1567–91.
[33] Ljung T, Hellstrom PM. Vasoactive intestinal peptide suppresses migrating myoelectric complex of rat small intestine independent of nitric oxide. Acta Physiologica Scandinavica 1999;165:225–31.
[34] Mondal MS, Nakazato M, Date Y, Murakami N, Yanagisawa M, Matsukura S. Widespread distribution of orexin in rat brain and its regulation upon fasting. Biochemical and Biophysical Research Communications 1999;256:495–9.
[35] Nakabayashi M, Suzuki T, Takahashi K, Totsune K, Muramatsu Y, Kaneko C, et al. Orexin-A expression in human peripheral tissues. Molecular and Cellular Endocrinology 2003;205:43–50.
[36] Nakade Y, Tsukamoto K, Pappas TN, Takahashi T. Central glucagon like peptide-1 delays solid gastric emptying via central CRF and peripheral sympathetic pathway in rats. Brain Research 2006;1111:117–21.
[37] Naslund E, Ehrstrom M, Ma J, Hellstrom PM, Kirchgessner AL. Localization and effects of orexin on fasting motility in the rat duodenum. American Journal of Physiology – Gastrointestinal and Liver Physiology 2002;282:G470–9.
[38] Novak CM, Jiang XL, Wang CF, Teske JA, Kotz CM, Levine JA. Caloric restriction and physical activity in zebrafish (Danio rerio). Neuroscience Letters 2005;383:99–104.
[39] Okumura T, Takeuchi S, Motomura W, Yamada H, Egashira S, Asahi S, et al. Requirement of intact disulfide bonds in orexin-A-induced stimulation of gastric acid secretion that is mediated by OX1 receptor activation. Biochemical and Biophysical Research Communications 2001;280:976–81.
[40] Ouedraogo R, Naslund E, Kirchgessner AL. Glucose regulates the release of orexin-A from the endocrine pancreas. Diabetes 2003;52:111–7.
[41] Park ES, Yi SJ, Kim JS, Lee HS, Lee IS, Seong JK, et al. Changes in orexin-A and neuropeptide Y expression in the hypothalamus of the fasted and high-fat diet fed rats. Journal of Veterinary Science 2004;5:295–302.
[42] Rodriguezmembrilla A, Martinez V, Jimenez M, Gonalons E, Vergara P. Is nitric-oxide the final mediator regulating the migrating myoelectric complex cycle. American Journal of Physiology – Gastrointestinal and Liver Physiology 1995;31:G207–14.
[43] Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1998;92:1, page following 696.
[44] Samson WK, Resch ZT. The hypocretin/orexin story. Trends in Endocrinology and Metabolism 2000;11:257–62.
[45] Satoh Y, Takeuchi T, Yamazaki Y, Okishio Y, Nishio H, Takatsuji K, et al. Mediators of nonadrenergic, noncholinergic relaxation in longitudinal muscle of the intestine of ICR mice. Journal of Smooth Muscle Research 1999;35:65–75.
[46] Smart D, Jerman JC, Brough SJ, Rushton SL, Murdock PR, Jewitt F, et al. Characterization of recombinant human orexin receptor pharmacology in a Chinese hamster ovary cell-line using FLIPR. British Journal of Pharmacology 1999;128:1–3.
[47] Tache Y, Maedahagiwara M, Turkelson CM. Central-nervous-system action of corticotropin-releasing factor to inhibit gastric-emptying in rats. American Journal of Physiology 1987;253:G241–5.
[48] Takahashi N, Okumura T, Yamada H, Kohgo Y. Stimulation of gastric acid secretion by centrally administered orexin-A in conscious rats. Biochemical and Biophysical Research Communications 1999;254:623–7.
[49] Takahashi T. Pathophysiological significance of neuronal nitric oxide synthase in the gastrointestinal tract. Journal of Gastroenterology 2003;38:421–30.
[50] Tanabe Y, Calland JF, Schirmer BD. Effects of peritoneal injury and endotoxin on myoelectric activity and transit. Journal of Surgical Research 2004;116:330–6.
[51] Tanaka T, VanKlompenberg LH, Sarr MG. Selective role of vagal and nonvagal innervation in initiation and coordination of gastric and small bowel patterns of interdigestive and postprandial motility. Journal of Gastrointestinal Surgery 2001;5:418–33.
[52] Tomita R, Tanjoh K, Fujisaki S, Fukuzawa M. The role of nitric oxide (NO) in the human pyloric sphincter. Hepato-Gastroenterology 1999;46:2999–3003.
[53] Tritos NA, Mastaitis JW, Kokkotou E, Maratos-Flier E. Characterization of melanin concentrating hormone and preproorexin expression in the murine hypothalamus. Brain Research 2001;895:160–6.
[54] Wolf G. Orexins: a newly discovered family of hypothalamic regulators of food intake. Nutrition Reviews 1998;56:172–3.
[55] Zheng J, Ariga H, Taniguchi H, Ludwig K, Takahashi T. Ghrelin regulates gastric phase III-like contractions in freely moving conscious mice. Neurogastroenterology and Motility 2009;21:78–84.