

Extrinsic Orexin-A in Lateral Hypothalamic Area Regulates Gastric Motility in Rats

Heling Hao¹, Feifei Guo¹, Xiangrong Sun¹, Yanling Gong², Luo

Xu¹✍

¹Department of Pathophysiology, Medical College of Qingdao University, Qingdao, Shandong 266021, PR China

²Department of Pharmacy, College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao, Shandong, PR China

Abstract: The orexin neuropeptide family consists of orexin-A and orexin-B, which are coded from the same prepro-mRNA. Although peripherally administered orexin-A abolishes small intestinal interdigestive contractions in rats, it still remains unclear whether orexin-A of lateral hypothalamic area(LHA) effects on the gastric motility in rats. We selected Wistar rats as our subjects. A stainless-steel injection cannula was implanted unilaterally into the LHA. A force transducer was embedded into the stomach to record circular muscle contractions in freely moving conscious rats. The gastric motility was monitored by administration of ghrelin into LHA. The changes of amplitude of constriction and frequency of gastric motility were monitored in conscious rats by a transducer. Subdiaphragmatic vagotomy was performed to elucidate the neural pathways of orexin-A. After microinjection of orexin-A into LHA 5-20 min later, Orexin-A dose-dependently increased the amplitude of contraction and accelerated frequency of gastric motility in the stomach. The effect of orexin-A on promoting the amplitude and frequency of gastric contraction disappeared after 0.5 μ g orexin-A +6.0 μ g SB-334867 mixture was microinjection into LHA. However, there was no significant changes of amplitude and frequency of gastric contraction while injection of orexin-A receptor antagonists SB-334867 alone. The stimulatory effect of orexin-A on gastric motility was abolished by subdiaphragmatic vagotomy. It is suggested that exogenous orexin-A of LHA may promote gastric motility, the effect may be accomplished through the LHA-vagus pathway.

Keywords: orexin-A; lateral hypothalamic area(LHA);gastric motility; SB-334867

Introduction

The orexins consist of orexin-A and orexin-B, also named hypocretin 1 and hypocretin 2. They are hypothalamic peptides produced in lateral hypothalamic neurons and released widely throughout the central nervous system(CNS) [1,2]. Orexin-A and orexin-B regulate homeostatic mechanisms of energy balance and metabolism through activation of two G-protein coupled receptors [2,3], orexin receptors 1 and 2 (OX1R and OX2R, respectively). Orexin-A has a wide variety of physiological functions, such as feeding, arousal, behavioral activity and energy homeostasis [2-7]. Recent studies have shown that orexin-A regulate gastrointestinal motility through the brain-gut axis [8]. The evidences suggest that central orexin-A plays a role in gastrointestinal motility through the vagal pathways [9]. Orexin-A excites the gastric-projecting vagal motor neurons [9]. OXR1 is highly expressed in the dorsal motor nucleus of vagus (DMV) [10]. Administration of orexin-A into the DMV increased intragastric pressure and antral motility in anesthetized rats [11], the effects induced by orexin-A were attenuated by vagotomy [12]. However, it is not clear whether hypothalamus administered orexin-A modulates the gastric motility in conscious rats. The objective of our studies was to determine whether

orexin-A administration into LHA affects gastric motility, and whether the effect of orexin-A is mediated by the vagal pathways.

Methods

Animals

Adult male Wistar rats (weighing 250–300 g) were obtained from Qingdao Institute for Drug Control (Shandong, China). The rats were housed under a reversed 12 h/12 h light/dark cycle and temperature (22–26 °C) controlled room. Standard laboratory chow pellets and tap water were available *ad libitum*. The study was approved by Animal Care and Use Committee at Qingdao University, and all procedures were performed in accordance with institutional guidelines.

Group of experiments

This study consisted of two parts: Part A (n = 60), to study the effects and potential mechanisms of orexin-A in the LHA on gastric motility. The rats were randomly divided into six groups (n = 10, each group): (a) 0.5 μ L NS group; (b) 0.05 μ g/0.5 μ L orexin-A group; (c) 0.5

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Luo Xu (Correspondence)



xu.luo@163.com



+86-532-82991713

$\mu\text{g}/0.5 \mu\text{L}$ orexin-A group; (d) $5.0\mu\text{g}/0.5 \mu\text{L}$ orexin-A group; (e) $6.0\mu\text{g}/0.5 \mu\text{L}$ SB-334867 group; (f) $6.0\mu\text{g}/0.5 \mu\text{L}$ SB-334867 + $0.5\mu\text{g}/0.5 \mu\text{L}$ orexin-A group; Part B (n = 56), to observe the effects of orexin-A in the LHA on gastric motility after subdiaphragmatic vagotomy. The rats were divided into six groups (n = 8, each group): (a) sham operation group; (b) simple subdiaphragmatic vagotomy group; (c) sham operation + $0.5\mu\text{L}$ NS group; (d) sham operation + $5.0\mu\text{g}/0.5\mu\text{L}$ orexin-A group; (e) subdiaphragmatic vagotomy + $0.5\mu\text{L}$ NS group; (f) subdiaphragmatic vagotomy + $5.0\mu\text{g}/0.5\mu\text{L}$ orexin-A group.

Animal surgery

After fasting for 18 h, the rats were anaesthetized with thiobutabarbital and placed in a stereotaxic apparatus (Narashige SN-3, Tokyo, Japan). A stainless-steel injection cannula was implanted unilaterally into the hypothalamus lateral area (administration site, bregma: P: 2.3-2.7 mm, L (R): 1.8-2.2 mm, H: 8.5-9.0 m)^[13]. The injection cannula (29 gauge) was connected to a syringe by a 10 cm piece of polyethylene tubing.

After a cannula was implanted, a midline laparotomy was performed. Briefly, the abdominal cavity was exposed and contractile force transducers were sutured onto the serosa of the gastric antrum, 0.5 cm caudal to the pyloric ring, to measure circular muscle motility^[14]. The lead wires of the force transducers were tunneled subcutaneously and exteriorised at the nape of the neck, protruding 2–3 cm through a small incision between the scapulae^[14]. Following this, the abdomen was closed with suture and the animals were allowed to recover for 4 days before administering chemicals.

To evaluate the mediation of the vagal nerve, subdiaphragmatic vagotomy was performed at the time of transducer implantation. Under the dissecting microscope, the lower part of the esophagus was exposed and anterior and posterior branches of the vagal nerves were incised above the hepatic and celiac branches, as previously reported^[15]. In sham operation rats, the vagal trunks were similarly exposed but not cut^[16]. Sham-operated rats served as the control. After the surgery, rats were housed individually, with access to a standard diet and tap water. Prior to the measurement of gastric motility, they were allowed to recover for 1 week.

Gastric motility recordings

Gastric motility was recorded on a polygraph (3066–23; Chengdu Precision Instruments, Chengdu, Sichuan, China). The rats, fasted overnight, were acclimated for 30 min at the recording area. After baseline motility had been recorded for 30 min, orexin-A or SB-334867 was injected via the cranial cannula, but during the experiment they could move around freely in their cages^[17]. For the control group, equal volumes of 0.9% saline were injected. The amplitude and frequency of gastric motility were measured. For each animal, recordings were made for 1–2 h per day, and lasted several days, with at least a 2 day period^[18].

Histological verification

Verification of the correct position of administration site was performed by injection of Pontamine sky blue ($2\mu\text{L}$) at the end of the recording of gastric motility^[14]. After perfusion and fixation of the brain, $50 \mu\text{m}$ frozen coronal sections were cut through the regions of the LHA, stained with Neutral Red and observed under a light microscope^[14]. Samples with incorrect locations were eliminated from the analysis.

Statistical analysis

Data are expressed as mean \pm SD. Percentage of the frequency and amplitude change was derived from the equation: frequency or amplitude change = (frequency or amplitude after microinjection - frequency or amplitude before microinjection) / frequency or amplitude before microinjection $\times 100\%$. Data were processed with SPSS 17.0 statistics software. Comparisons were made between groups by either Student's t-test or two-way ANOVA followed by post hoc Bonferroni tests for comparison among means. $P < 0.05$ was considered as statistically significant.

Results

Effects of orexin-A administration to the LHA on gastric motility

After administration of orexin-A in the LHA, the amplitudes and frequencies of the gastric contraction significantly increased compared with the saline group (Fig. 1). The increase in amplitude after injection of $0.05 \mu\text{g}$, $0.5 \mu\text{g}$, $5.0 \mu\text{g}$ orexin-A had a latency of 5 min and lasted for around 5–10, 10–15, 10–20 min respectively (Fig. 1). However, after the administration of $1 \mu\text{L}$ of the mixture of $0.5\mu\text{g}$ orexin-A and $6.0\mu\text{g}$ SB-334867 absolutely blocked the effects of orexin-A (Fig. 1). Meanwhile, when given SB-334867 or saline alone, the amplitude showed no significant change.

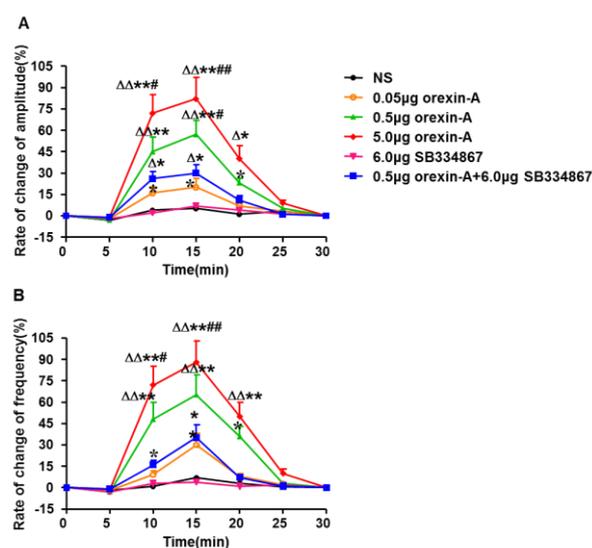


Figure 1. Effects of orexin-A on the amplitudes(A) and frequencies(B) of gastric motility in the LHA

Notes: * $P < 0.05$, ** $P < 0.01$ versus NS group; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ versus $0.05\mu\text{g}$

Effects of orexin-A in the LHA on gastric motility after subdiaphragmatic vagotomy

After subdiaphragmatic vagotomy 1 week, injection of orexin-A into the LHA five min later the amplitude and frequency of gastric contractions of rats significantly decreased compared with sham operation + NS group ($P < 0.05$, Fig. 2), but no difference between sham operation group and sham operation + NS group. When 5.0 μg orexin-A was administered into the LHA in subdiaphragmatic vagotomy rats, the amplitude and frequency of gastric contractions dramatically reduced compared with sham operation + orexin-A group, there was no significant change in subdiaphragmatic vagotomy group, subdiaphragmatic vagotomy + NS group and subdiaphragmatic vagotomy+orexin-A group (Fig. 2)

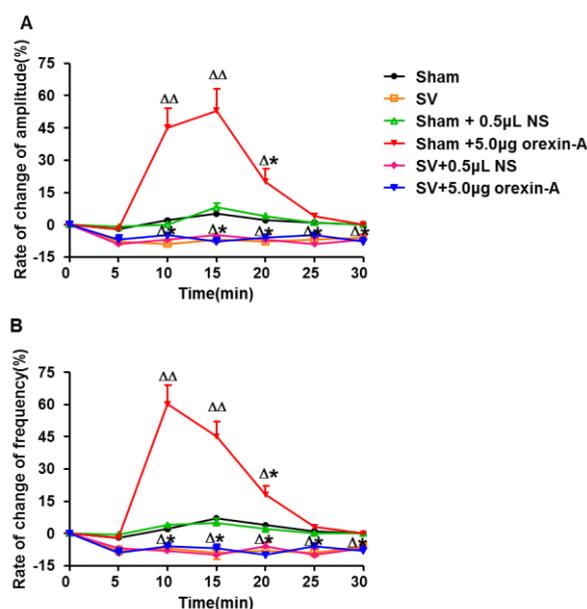


Figure 2. Effects of orexin-A in the LHA on the amplitudes and frequencies of gastric motility after subdiaphragmatic vagotomy.

Notes: Note: * $P < 0.05$, compared with sham operation; $\Delta P < 0.05$, $\Delta\Delta P < 0.01$, compared with Sham + 0.5 μL NS. Sham: sham operation; SV: subdiaphragmatic vagotomy.

Discussion

The present study has demonstrated that orexin-A administration to the LHA can promote gastric motility, which could be absolutely eliminated by pretreatment with orexin-A receptor antagonist, SB-334867. This suggests that orexin-A neurons in the LHA participating in the regulation of gastric motility. Research indicates that postprandial-like motility pattern induced by orexin-A was abolished by atropine and vagotomy^[10]. Thus, we speculate that exogenous orexin-A may act on the LHA to regulate gastric motility via the vagus pathway.

The hypothalamus plays an important role in regulating food intake and energy metabolism with dense neuronal connections to higher-order brain regions^[19]. Discrete hypothalamic regions that have been identified

as contributing to the neural control of ingestive behavior include the ventromedial hypothalamic nuclei (VMH), the arcuate nucleus (ARC), the paraventricular nucleus (PVN) and LHA^[20]. As there are LHA cells containing various orexigenic neuropeptides, the LHA becomes an important region involved in the control of ingestive behavior, closely associated with the initiation and regulation of feeding^[20].

In the brainstem, the dorsal vagal complex (DVC) consists of the AP, NTS and DMN. These nuclei receive vagal afferent inputs from the proximal gastrointestinal tract and also form gastric vagal efferent^[11]. The hypothalamus and DVC play an important role in regulating gastric motility and feeding behavior. The brainstem nuclei could control gastric motility, feeding behavior, and energy homeostasis^[21]. The hypothalamus has complex, interactive connections with brainstem nuclei, and it could regulate the synthesis and release of neurohypophysial hormones through both nervous and humoral routes^[21-23]. Our results show that orexin-A injection in the LHA activated neurons in the hypothalamic nuclei and the DVC of the brainstem to promote gastrointestinal activities. No difference in gastric motility was observed before and after orexin-A injection in subdiaphragmatic vagotomy rats, indicating that pretreatment of subdiaphragmatic vagotomy abolishes the promoted gastric contractions induced by orexin-A administration. This finding indicated that the LHA-DVC-vagus-gastrointestinal pathway may be involved in how orexin-A in the LHA mediates gastric motility.

In summary, exogenous orexin-A administered to the LHA could modulate gastric motility, and the DVC may participate in the regulatory process. Our study provides a new avenue for the diagnosis of gastrointestinal diseases and potential drug development for the treatment of such disease.

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