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Ghrelin and electrical stimulating the lateral hypothalamus area regulated the discharges of gastric distention neurons via the dorsal vagal complex in cisplatin-treated rats

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ABSTRACT

Objective: Cisplatin is an important antineoplastic drug and has side effects such as nausea, vomiting, and dyspepsia. The detailed mechanisms for its side effects are yet not well be illustrated. Our purpose was to investigate the discharges of gastric distention (GD) sensitive neurons regulated by ghrelin and electrical stimulation of the lateral hypothalamus area (LHA) via the dorsal vagal complex (DVC) in cisplatin-treated rats.
Materials and methods: Extracellular discharge recording was performed to observe the effects of ghrelin and electrical stimulation of the LHA on discharges of GD neurons in the DVC.
Results: GD neurons were recorded in DVC in saline-treated and cisplatin-treated rats and identified as GD-excitatory (GD-E) neurons, which are excited by gastric distension, and GD-inhibitory (GE-I) neurons, which are inhibited by gastric distension. Microinjection of ghrelin into the DVC increased the firing frequency of most GD neurons, while the ratios of excited GD-E and GD-I neurons in cisplatin-treated rats were significantly lower than those in saline-treated rats. The excitatory effect of ghrelin was eliminated completely by DVC pretreatment with ghrelin receptor antagonist [D-Lys-3]-GHRP-6. After electrical stimulation of the LHA, the firing frequency of these neurons significantly increased. This excitatory effect was weaker in cisplatin-treated rats than in saline-treated rats and could be partly blocked by DVC pretreatment with [D-Lys-3]-GHRP-6.
Conclusion: GD neurons in the DVC could be excited by microinjecting ghrelin into the DVC and electrical stimulation of the LHA, respectively. The excitatory effect was attenuated by cisplatin injected intraperitoneally.

1. Introduction

A variety of feedback signals in the body can affect feeding, including gastrointestinal peptides that could transmit the information into the brain to regulate ingestion (Rusin et al., 2013; King, 2013). Therefore, localization of the gastric sensation in the brain is important for understanding the neural mechanisms of satiation, among which gastric distention during eating has a role in the regulation. Previous studies have shown that in rodents, the hypothalamus and brain stem are regions involved in mediating gastric distention-induced satiety (Hellström et al., 2004). The decreased sensitivity of neurons to these signals may lead to uncontrolled eating and obesity (Powley, 2000). Intragastric balloon insertion, which is used to simulate the rate of digestion and emptying of mixed liquid foods, can lead to a reduction in food intake and an increase in blood pressure. It has been found that gastric distension can activate the hypothalamus, nucleus of the solitary tract, the amygdala, the thalamus, the cerebellum, and the cerebral cortex (Min et al., 2011). The afferent signals from the periphery (gastrointestinal tract, fatty tissue, and so on) send out the impulses through the complex neural network to control the food intake and energy metabolism (Petrovich, 2013; Perry and Wang, 2012). The LHA, an important functional area of autonomic nervous system, controls feeding behavior (Stuber and Wise, 2016).

Ghrelin was secreted by the endocrine cells in the oxyntic glands of the fundus stomach (Kojima et al., 1999). Ghrelin was the only endogenous ligand of growth hormone secretagogue receptor (GHSR-1a),

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and the GHSR-1a was located in both the central nervous system and the periphery (Zigman et al., 2006; Bron et al., 2013). Following the effect of ghrelin on release of growth hormone from the anterior pituitary growth hormone cells, it was reported that ghrelin also had a role in promoting appetite through the hypothalamus (Korbonits and Grossman, 2004; Kirsz and Zieba, 2011). Sakata et al. also reported that ghrelin was a hormone that influenced many physiological processes and behaviors, such as food intake, insulin, and growth hormone release (Sakata et al., 2009). Ghrelin increases vagally-mediated gastric activity by central sites of action (Swartz et al., 2014). Ghrelin can enhance appetite in rodents and humans while administrated peripherally, although most of the peptides released from the brain play a role only when injected into the brain (Szentirmai et al., 2007; Darmani and Ray, 2009). Not long ago, we revealed that the LHA sent ghrelin fibers to the DVC, which involved in the gastric motility regulation in the cisplatin-treated rats (Gong et al., 2017a).

Cisplatin is one of the most important antineoplastic drugs used for a variety of malignant tumors. Chemotherapy-induced nausea and vomiting (CINV), one of the most dreaded side effects associated with chemotherapy, plays a significant role in cancer patients' morbidity and quality of life and renders difficulty in continuation of chemotherapy (Ando et al., 2016; Eisenberg et al., 2003). The emetic process is mainly controlled by the medullary dorsal vagal complex (DVC) in the brain stem, which includes the area postrema (AP), the dorsal motor nucleus of the vagus (DMV), and the nucleus tractus solitarius (NTS) (Welkenhuysen et al., 2008). These areas receive and process various emetic stimuli and also generate efferent signals to the vasomotor, respiratory, and salivary centers, as well as the cranial nerves VIII and X, which result in nausea and vomiting (Gad Elhak et al., 2004). The LHA regulates food intake by modulating the DVC (Welkenhuysen et al., 2008; Zhang and Tang, 2002; Hou et al., 2006).

In our previous study, we have recorded discharges of GD neurons in the DVC in rats. Is there any change in discharges of GD neurons in the cisplatin-treated rats? Whether ghrelin microinjection and electrical stimulating the LHA affect the discharges of GD neurons via the DVC remains unrevealed. Therefore, in the present study, nuclei microinjection, electrical stimulation, and electrophysiology were used to record the spontaneous discharge activities of neurons in the DVC. The effects of ghrelin in the DVC and electrical stimulation of the LHA on the activities of GD neurons in the DVC in the cisplatin-treated rats were observed and recorded.

2. Material and methods

2.1. Animals and treatments

Adult male Wistar rats, which were purchased from Qingdao Daren Fortune Animal Technology Co., Ltd., weighing 260–350 g, were used in this experiment. The rats were housed in a separate cage individually, and fed with standard animal chow except where otherwise indicated. The temperature and the relative humidity of the animal room were maintained at 24 ± 1 °C and 60 ± 5%, respectively. The animal room was lighted from 8:00 am to 8:00 pm. One week of adaptive feeding was carried out before the experiment. All animal experiments were in conducted in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Council of Europe No 123, Strasbourg 1985).

2.2. Gastric surgery

The rats were fasted for 15–20 h before the operation and anesthetized with thiobutabarbitol (100 mg kg^{-1} i.p., Sigma, USA). A radio telemetry unit (PA-C40; Data Sciences International, St. Paul, MN) was implanted to monitor the heart rate of rats (Ulrich-Lai et al., 2010). A latex balloon was placed in the stomach by surgery and connected to a 5 mL syringe. The saline was injected into the stomach with the syringe and the gastric wall was stimulated. The incision was sutured after the operation. Three to five mL 37 °C saline was inflated into the balloon with a rate of 0.5 mL s^{-1} and maintained for 20 s (Appia et al., 1986). The pylorus was ligatured to avoid the duodenal reflux, which might affect the gastric volume. The rat was then positioned on a stereotaxic frame after the gastric surgery (Narashige SN-3, Tokyo, Japan), and a cranial surgery was performed for extracellular discharge recording.

2.3. Extracellular discharge recording and microinjection of ghrelin into DVC

Sixty rats were randomly divided into two groups: the cisplatin group and the normal saline group. A dose of cisplatin (6 mg/kg, Qilu Pharmaceutical Co. Ltd, Hainan, China) was intraperitoneally injected (i.p.) into the rats in the cisplatin group. A same volume (4 mL/kg) of 0.9% saline was intraperitoneally injected (i.p.) into the rats in the saline group. The rats were anaesthetized with thiobutabarbitol (100 mg kg^{-1} i.p., Sigma, USA) and placed on a stereotaxic frame. During the whole experiment, the heart rate of the rats was monitored. After the cranial surgery was performed, a four-barrel glass microelectrode (total tip diameter 3–10 μm, resistance 5–15 MΩ) was advanced in 10 μm steps with the aid of a hydraulic micropositioner into the area of DVC (bregma: P: 13.12–13.68 mm, L(R): 0.4–0.5 mm, H: 6.8–7.5 mm) for extracellular discharge recording and micro-pressure injection (Sakurazawa et al., 2013). The recording glass microelectrode was filled with 0.5 M sodium acetate and 2% pontamine sky blue, while the other three barrels connected with a 3-channel pressure injector filled with 15 nmol L^{-1} solution of ghrelin (Abcam, USA), 150 nmol L^{-1} solution of ghrelin receptor antagonist [D-Lys-3]-GHRP-6 (Abcam, USA), and normal saline, respectively. Drugs of 3 nl were ejected on the surface of firing cells with short pulse gas pressure (100–1500 ms, 3.0–35.0 psi) (Jhamandas et al., 1989). Given the possibility of receptor desensitization from the first application, we have observed the effect of sequential ghrelin applications with a 4 min, 5 min, and 6 min interval, respectively. The result revealed that there was no significant difference between these two sequential ghrelin applications. Therefore, we applied ghrelin again with an interval of at least 6 min.

The discharge signal was input through a microelectrode amplifier, and the RM6240B biological signal processing system was used to analyze the discharge frequency.

After 3–5 mL saline was inflated into the balloon with a rate of 0.5 mL s^{-1} and maintained for 20 s, the changes of neuronal firing activity were observed. The basal firing frequency was determined by the average frequency of 120 s baseline data before treatment. The maximal change of frequency within 50 s following drug application was considered a drug effect, and a change of at least 20% of basal firing frequency during drug application was considered significant. A neuron was identified as a GD-sensitive neuron if its mean firing frequency changed via GD by at least 20% from the mean basal firing level. The GD-sensitive neurons were further classified into GD-excitatory (GD-E) neurons and GD-inhibitory (GD-I) neurons according to the spontaneous discharge increased or decreased with GD, respectively (Fig. 1).

2.4. Electrical stimulation of the LHA

Sixty-four rats were randomly divided into two groups: the cisplatin group and the normal saline group. A dose of cisplatin (6 mg/kg, Qilu Pharmaceutical Co. Ltd, Hainan, China) was intraperitoneally injected (i.p.) into the rats in the cisplatin group while a same volume (4 mL/kg) of 0.9% saline was intraperitoneally injected (i.p.) to the rats in the saline group. The rats were anaesthetized with thiobutabarbitol (100 mg kg^{-1} i.p., Sigma, USA) and then placed on a stereotaxic framer. During the whole experiment, the heart rate of rats was always

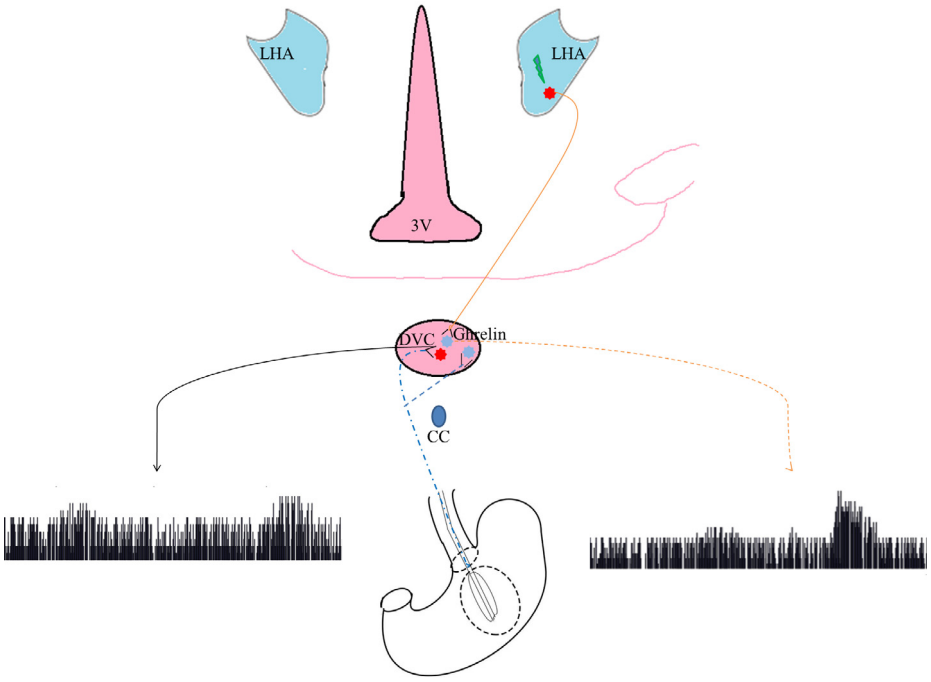

Fig. 1. Summary for ghrelin and electrical stimulating on the LHA regulated the discharges of gastric distention neurons via dorsal vagal complex. When the stomach was stretched, some of the neurons in DVC changed their discharge frequency; that is, GD neurons (the blue dotted lines). While ghrelin was administered into the DVC, the discharge frequency of GD neurons changed (the black solid lines). Electrically stimulating the LHA (the orange solid lines) could excite the ghrelin-responsive GD neurons in the DVC (the orange dotted lines).

monitored. A monopolar stimulation electrode (RH NE-100 1 mm × 50 mm; David Kopf Instruments, Tujunga, CA, USA), insulated with epoxy resin to within 200 μm of the tip, was inserted into the LHA (bregma: P: 2.0–2.8 mm, L(R): 1.8–2.3 mm, H: 8.5–9.0 mm). A four-barrel glass microelectrode, as described above, was inserted into the DVC. Electrical stimulation (ES) of the LHA was performed during the recording of the DVC GD neuron discharges in rats. The parameters were as follows: wave width: 0.5 ms; intensity: 20 μA; frequency: 50 Hz and lasting for 10 s (Avetisyan et al., 2004). The discharge activities of GD neurons in the DVC, which were responsive to ghrelin, were observed. The rats in the sham stimulation group had only buried electrodes with no electricity. Given that the receptor desensitization/depression was independent of any effect of ghrelin, we have elicited a continuous ES with a 5 min, 6 min, and 7 min interval, respectively, and there was no significant difference between any two successive ES. Therefore, we elicited ES again with an interval of at least 7 min (Fig. 1).

2.5. Anatomical verification

At the end of the experiment, a direct current (10 μA, 20 min) was passed through the electrode to form an iron deposit of pontamine sky blue to check the position of the recording electrode. Fifty μm series coronal sections were obtained after perfusion and fixation performed. The sections were observed under a microscope and the recording site was labeled by pontamine sky blue (Fig. 2). If the recorded site was not in the DVC, the experimental data was eliminated from the statistical analysis.

2.6. Identification of ghrelin expression in the LHA

2.6.1. Polymerase chain reaction (PCR)

After fasted for 24 h, the rats were sacrificed and the brains were removed immediately. Total RNA was extracted from the LHA using a TRIzol Plus RNA Purification Kit (Invitrogen, CA, USA), and reverse transcribed using a First Strand cDNA Synthesis Kit (Pharmacia Biotech, Piscataway, NJ, USA). The resulting cDNA was subjected to PCR amplification with 2 μmol L^{-1} each of the sense and antisense primers and 2.5 units of Pyrobest DNA polymerase (Takara Shuzo, Shiga, Japan). PCR primer for rat ghrelin (347 bp) was 5′-TTG AGC CCA GAG CAC CAG AAA-3′ (sense) and 5′-AGT TGC AGA GGA GGC AGA AGC T-3′ (antisense). PCR was conducted in a reaction volume of 25 μl for 33 cycles as follows: denaturation at 94 °C for 60 s, annealing at 64 °C for 70 s, and extension at 72 °C for 90 s. PCR products were visualized with ethidium bromide after electrophoresis on 2% agarose gel.

2.6.2. Western blotting (WB)

Tissue samples were lysed and homogenized in lysis buffer on ice for 30 min, and then cleared by centrifugation at 14,000 rpm for 15 min at 4 °C. 50 μg of proteins were fractionated on Tris-Tricine gradient gels, transferred to nitrocellulose membrane, and blocked with 5% milk in TBST for 1 h. Blots were incubated with specific rabbit anti-rat ghrelin IgG (1:1000; Abcam, USA) overnight at 4 °C. After washing five times in TBST for 10 min, blots were incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (1:2000; New England Biolabs) for 1 h at room temperature. A chemiluminescent peroxidase substrate (ECL, Amersham Biosciences) was applied and the membranes were exposed briefly to X-ray film.

2.7. Statistical analysis

Data of each group were described as mean ± SD. SPSS 17.0 and Prism 3.0 statistical software were used for data processing. Paired and non-paired Student's *t*-test was used to check the difference in treatment of the two groups. Significant differences were considered at $P < 0.05$.

3. Results

3.1. Effects of ghrelin in the DVC on the discharge activities of GD neurons in cisplatin-treated rats

The GD-sensitive neurons were recorded in the DVC of rats in saline-treated and cisplatin-treated groups. There was no significant difference in the proportion of GD-E and GD-I neurons. The discharge frequency between the two groups also had no significant difference.

Seventy-two (58.54%) GD-E neurons and 51 (41.46%) GD-I neurons were recorded in the DVC of the saline-treated rats. The firing

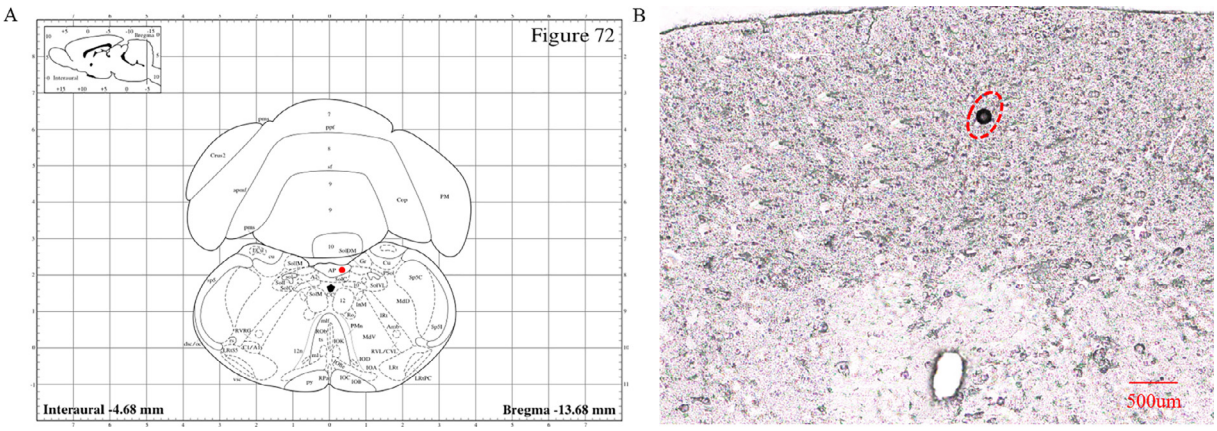

Fig. 2. Anatomical verification of the recording site. A red dot indicating extracellular recording site in the brain (A) and a black dot showing pontamine sky blue in the electrophysiological recordings site by a typical photomicrograph of the coronal section of rat brain (B). Bar: 500 μm.

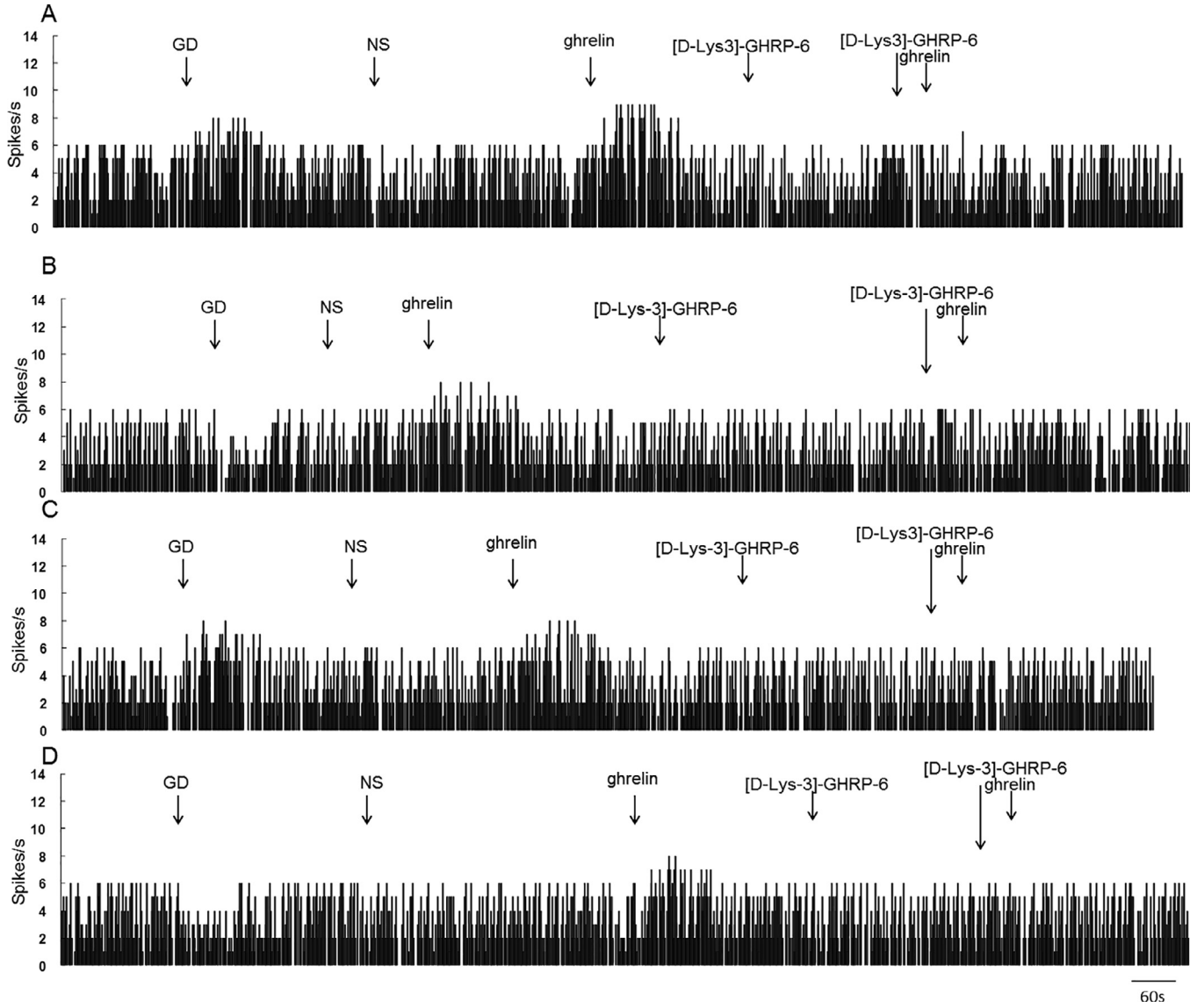

Fig. 3. Effects of ghrelin in DVC on the discharge activities of GD neurons in saline-treated and cisplatin-treated rats. The GD-sensitive neurons were both recorded in the DVC of rats in two groups. Both GD-E and GD-I neurons were excited after microinjection of ghrelin into DVC of saline-treated (Fig. 3A and B respectively) and cisplatin-treated rats (Fig. 3C and D respectively). This excitatory effect could be completely blocked by DVC pretreatment with ghrelin receptor antagonist [D-Lys-3]-GHRP-6 in the saline group (Fig. 3A and B) and the cisplatin group (Fig. 3C and D), respectively. “NS” was normal saline. Scale bars, 60 s.

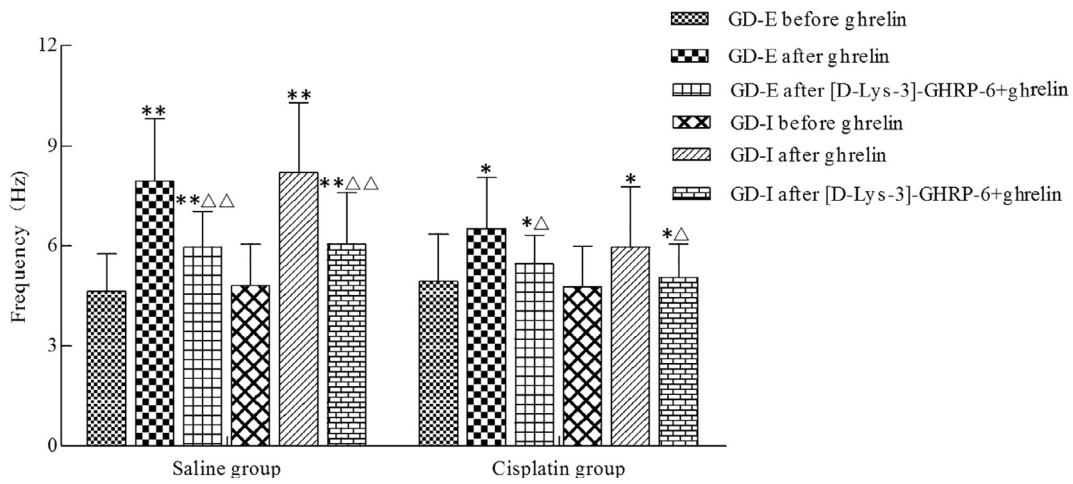

Fig. 4. Effects of ghrelin in DVC on the discharge frequency of GD neurons in saline-treated and cisplatin-treated rats. The firing frequency of GD-E and GD-I neurons increased after microinjection of ghrelin into DVC in saline-treated and cisplatin-treated rats. This excitatory effect could be completely blocked by the DVC pretreatment with ghrelin receptor antagonist [D-Lys-3]-GHRP-6 Compared with before ghrelin, *P < 0.05, **P < 0.01 Compared with after ghrelin, △P < 0.05, △△P < 0.01.

frequency neurons increased in 61.31% GD-E and 74.58% GD-I (Fig. 3A and B, Fig. 4) after microinjection of ghrelin into the DVC. These results suggest that the DVC ghrelin could excite most GD-E and GD-I neurons. The firing frequency of GD-E neurons increased from 4.63 ± 1.13 Hz to 7.94 ± 1.87 Hz and the firing frequency of GD-I neurons increased from 4.81 ± 1.23 Hz to 8.21 ± 2.09 Hz. This excitatory effect could be completely blocked by the DVC pretreatment with ghrelin receptor antagonist [D-Lys-3]-GHRP-6. The firing frequency of GD-E neurons decreased from 7.94 ± 1.87 Hz to 5.96 ± 1.08 Hz and the firing frequency of GD-I neurons decreased from 8.21 ± 2.09 Hz to 6.04 ± 1.55 Hz (Fig. 3A and B).

One hundred and twelve GD neurons were recorded in the DVC of cisplatin-treated rats, of which 53 (47.32%) were GD-E and 59 (52.67%) were GD-I. 45.67% GD-E and 47.18% GD-I neurons were excited after microinjection of ghrelin into the DVC (Fig. 3C and D, Fig. 4). The firing frequency of GD-E and GD-I neurons increased from 4.94 ± 1.41 Hz to 6.52 ± 1.53 Hz and 4.77 ± 1.22 Hz to 5.96 ± 1.81 Hz, respectively. This excitatory effect could be completely blocked by the DVC pretreatment with ghrelin receptor antagonist [D-Lys-3]-GHRP-6. The firing frequency of GD-E decreased from 6.52 ± 1.53 Hz to 5.46 ± 0.86 Hz and the firing frequency of GD-I decreased from 5.96 ± 1.81 Hz to 5.06 ± 0.99 Hz (Fig. 3C and 3D).

The firing frequency varied rates of GD-E and GD-I neurons that could be excited by ghrelin in cisplatin-treated rats were significantly lower than that in saline-treated rats. In addition, the firing frequency varied rates of GD neurons were also significantly lower than that in saline-treated rats (Fig. 5).

This study suggested that exogenous ghrelin could regulate the gastrointestinal signal transduction through the DVC and that this effect could be reduced by cisplatin.

3.2. Effects of electrical stimulation of the LHA on the discharge activities of GD neurons in cisplatin-treated rats

Electrical stimulation of the LHA was performed to reveal the regulation role of LHA has in ghrelin on the discharges of GD neurons in this experiment. After electrical stimulation of the LHA in saline-treated rats, the firing frequency of these GD-E and GD-I neurons that were excited by ghrelin increased from 4.38 ± 1.14 Hz to 11.68 ± 2.04 Hz and 4.22 ± 1.13 Hz to 11.92 ± 2.11 Hz, respectively (Fig. 6A and 6B, Fig. 7). This excitatory effect could be partly blocked by the DVC pretreatment with ghrelin receptor antagonist [D-Lys-3]-GHRP-6. The firing frequency of GD-E neurons decreased from 11.68 ± 2.04 Hz to 7.56 ± 1.49 Hz and the firing frequency of GD-I neurons decreased from 11.92 ± 2.11 Hz to 8.06 ± 1.88 Hz (Fig. 6A and B).

In cisplatin-treated rats, electrical stimulation of the LHA excited GD-E and GD-I neurons with firing frequency increased from 4.15 ± 1.08 Hz to 9.26 ± 1.97 Hz and 4.35 ± 1.23 Hz to 8.97 ± 2.01 Hz, respectively (Fig. 6C and D, Fig. 7). DVC pretreatment with ghrelin receptor antagonist [D-Lys-3]-GHRP-6 partly blocked this excitatory effect. The firing frequency of GD-E neurons decreased from 9.26 ± 1.97 Hz to 6.78 ± 1.22 Hz and the firing frequency of GD-I neurons decreased from 8.97 ± 2.01 Hz to 6.23 ± 1.59 Hz (Fig. 6C and D). The firing frequency varied rates in cisplatin-treated rats, which were excited by electrical stimulation of the LHA, were significantly lower than that in the saline-treated rats (Fig. 8).

3.3. Ghrelin expression in the LHA

In order to identify whether ghrelin expresses in the LHA, we conducted PCR and WB in this experiment. The PCR result revealed that there was a band with an expected length of 347 bp for ghrelin (Fig. 9A). Furthermore, a band at 48 kDa for ghrelin in the WB confirmed the expression of ghrelin in the LHA (Fig. 9B).

4. Discussion

The present study investigated the effect of the DVC ghrelin and electrical stimulation of the LHA on the discharge activities of gastric distention neurons in cisplatin-treated rats. These results revealed that GD-sensitive neurons were recorded in the DVC. There were no statistical differences in the ratios and the changes of firing frequency of GD-E and GD-I neurons between the cisplatin-treated rats and the saline-treated rats. After microinjection of ghrelin into the DVC, the firing frequency of GD-E and GD-I neurons increased significantly, which could be eliminated completely by the DVC pretreatment with [D-Lys-3]-GHRP-6. However, the ratios of GD-E and GD-I neurons, which could be excited by DVC ghrelin in cisplatin-treated rats, were significantly lower than that in saline-treated rats. Furthermore, electrical stimulation of the LHA excited the above ghrelin-responsive GD-E and GD-I neurons, which could be partly blocked by the DVC pretreatment with [D-Lys-3]-GHRP-6. The excitatory effect of electrical stimulation was weaker in cisplatin-treated rats than in saline-treated rats. These results suggested that ghrelin in the DVC and electrical stimulating on the LHA could change the discharge of GD neurons via ghrelin receptor in the

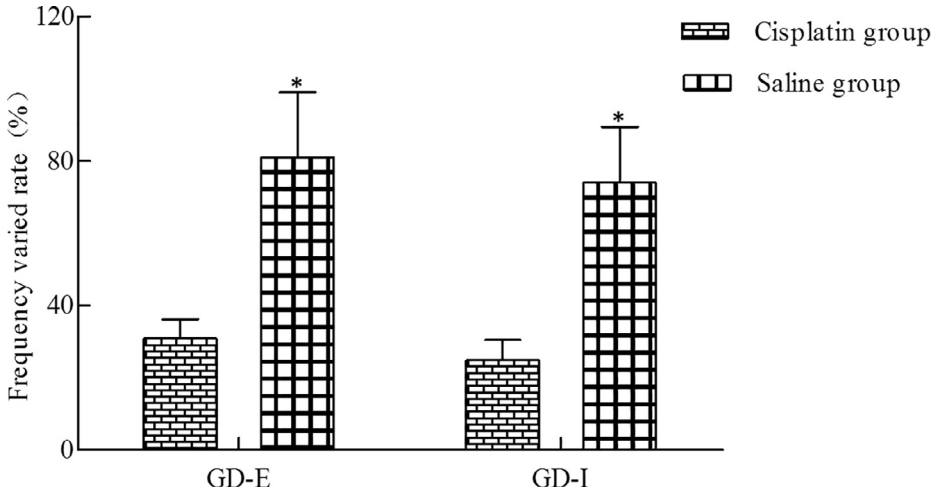

Fig. 5. Effects of ghrelin in DVC on the firing frequency varied rates of GD neurons in rats. The firing frequency varied rates of GD-E and GD-I neurons that could be excited by ghrelin in the DVC in cisplatin-treated rats were significantly lower than that in saline-treated rats. Compared with saline-treated group, *P < 0.01.

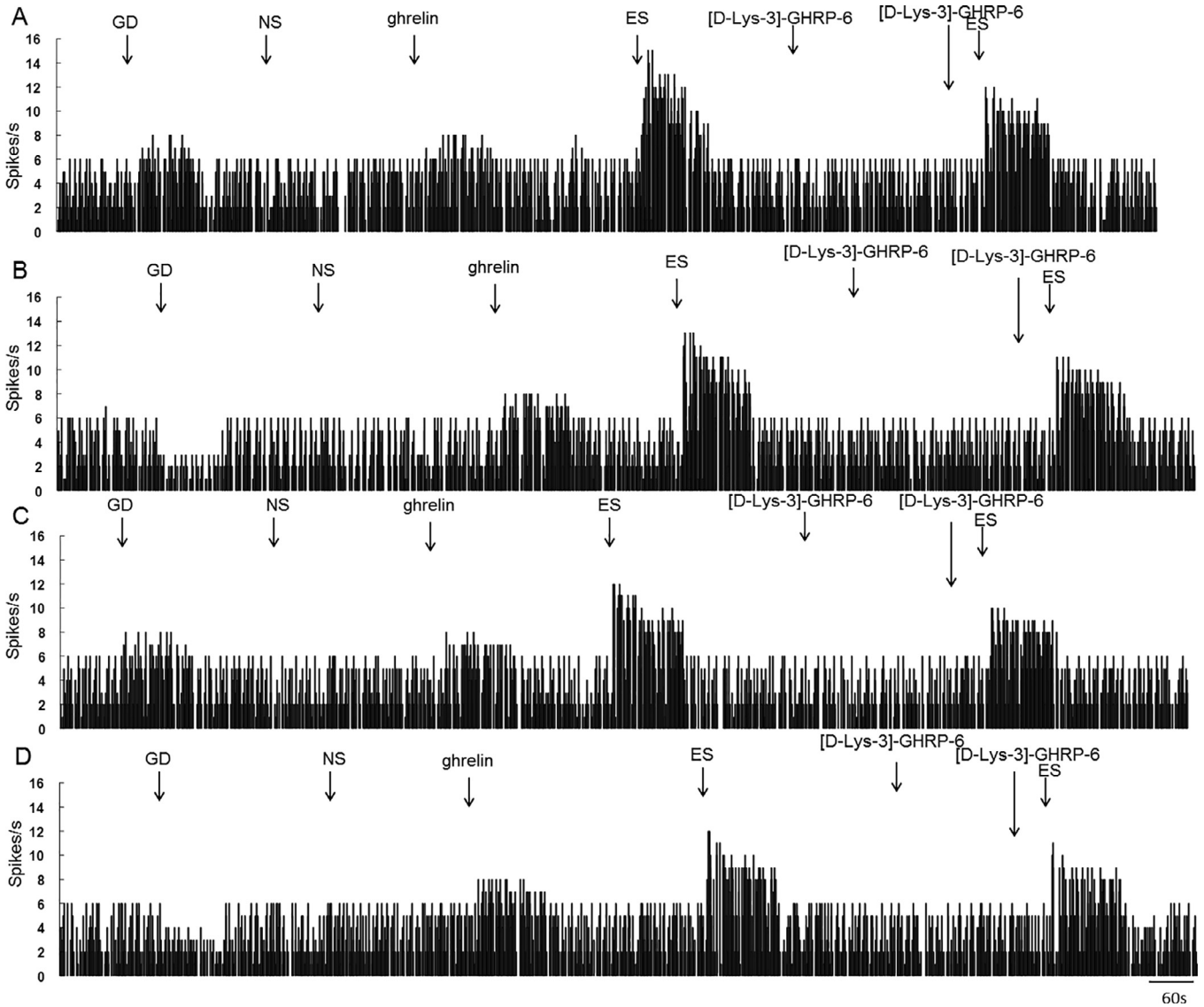

Fig. 6. Effects of electrical stimulation of LHA on the discharge activities of GD neurons in saline-treated and cisplatin-treated rats. GD-E and GD-I neurons were both activated after ghrelin administration into the DVC in two groups. Electrical stimulation of LHA could excite GD-E and GD-I neurons in the saline-treated rats (Fig. 6A and B respectively) and the cisplatin-treated rats (Fig. 6C and D) which were excited by ghrelin. This excitatory effect could be partly blocked by DVC pretreatment with ghrelin receptor antagonist [D-Lys-3]-GHRP-6 in saline-treated rats (Fig. 6A and B) and cisplatin-treated rats (Fig. 6C and D), respectively. Scale bars, 60 s.

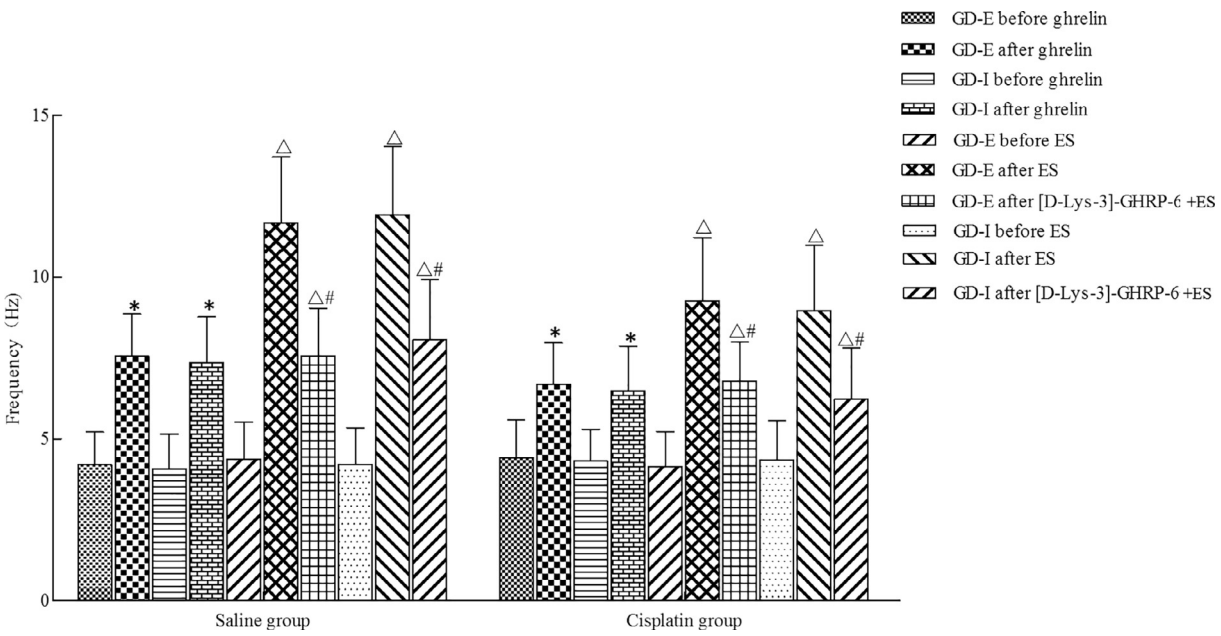

Fig. 7. Effects of electrical stimulation of LHA on the discharge frequency of GD neurons in saline-treated and cisplatin-treated rats. The firing frequency of GD-E and GD-I neurons increased after microinjection of ghrelin into the DVC of saline-treated and cisplatin-treated rats. Electrical stimulation of LHA could excite GD-E and GD-I neurons, which were excited by ghrelin in the two groups of rats. This excitatory effect could be partly blocked by the DVC pretreatment with ghrelin receptor antagonist [D-Lys-3]-GHRP-6. Compared with before ghrelin, * $P < 0.01$ Compared with before ES, △ $P < 0.01$ Compared with after ES, # $P < 0.01$.

DVC. Cisplatin attenuated the regulation process. The detailed mechanism needs to be investigated further.

Coordination of neuronal activity forms a complex network in the central nervous system. In order to understand better the temporal and spatial information of the neural network, it is necessary to adopt high-resolution monitoring technology. It was reported that electrophysiological extracellular recording is one of the important methods for high-resolution recording of nerve tissue (Imfeld et al., 2008). This method can provide information about the recording of neuronal discharge (output) and synaptic activity (input). With the deepening of the research, it is hoped that the data of extracellular electrophysiological recordings can be better explained on the neural network activity level. Extracellular electrophysiological results, combined with the morphological and anatomical information of the site, can help us better understand the input and output functions of the neural network information transformation. In this study, extracellular electrophysiological method was used to record the discharge activities of GD neurons in the DVC, which provided a new way for the DVC to participate in the regulation of gastric function.

Cisplatin was used for cancer chemotherapy, generating nausea, vomiting, anorexia and other behaviors indicative of malaise (Gad Elhak et al., 2004). Chemotherapy administration causes the release of several mediators by stimulating the enterochromaffin cells in the intestinal mucosa. These mediators bind to the specific receptors in vagal primary afferent neurons. The projection of vagal stimulus in the central nervous system leads to the emesis reflex. In rats, cisplatin decreased food intake and induced an increase of kaolin cumulative consumption (Gong et al., 2017b). The DVC is an important site through which peripheral afferent information from the gastrointestinal tract enters the brain. The DVC can sense the emetic substance in the blood, and relay the signal to nucleus tractus solitaries for information integration (Zhu et al., 2002). Therefore, the role of the DVC as a chemoreceptor is particularly important. Application of 5-HT antagonist in the whole body or in the DVC can alleviate the vomiting reaction (Pirnik et al., 2011). Are there any other mediators involved in the pathogenesis of emesis? In our previous study, we have revealed that ghrelin in the DVC participates in the regulation of gastric motility in rats (Gong et al., 2017a). More interestingly, we recorded a large

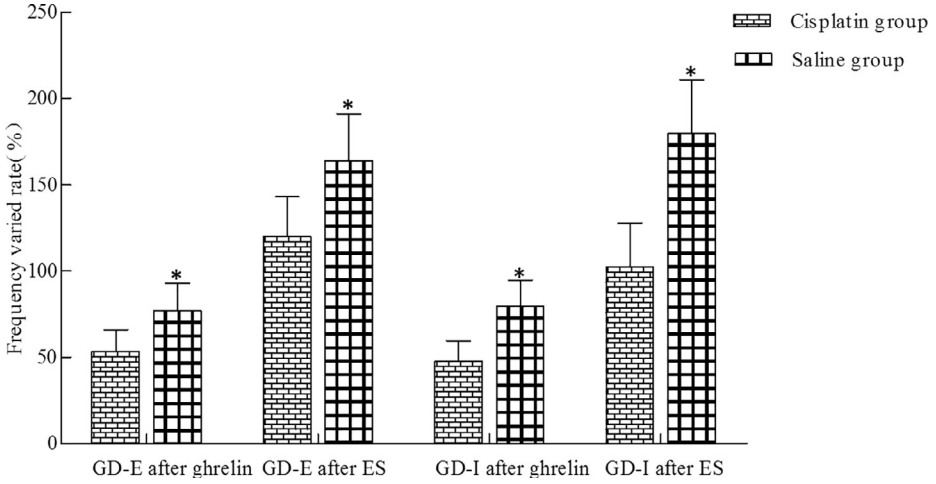

Fig. 8. Effects of electrical stimulation of LHA on the firing frequency varied rates of GD neurons in rats. The firing frequency varied rates of neurons in cisplatin-treated rats, which were excited by DVC ghrelin and the electrical stimulation of the LHA, was significantly lower than that of the saline-treated rats. Compared with pretreatment, * $P < 0.01$.

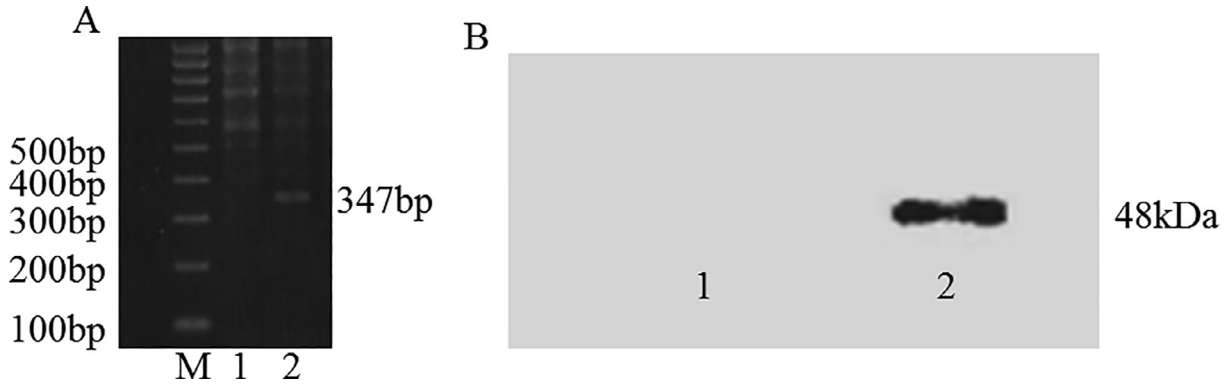

Fig. 9. Expression of ghrelin in the LHA of rats by PCR (A) and WB (B). (A): Expression of ghrelin mRNA in the LHA. M: markers; 1: LHA; 2: loading buffer. (B): Expression of ghrelin protein in the LHA. 1: no subject 2: LHA.

number of GD-sensitive neurons in the DVC, which suggested that the DVC could accept the gastrointestinal afferent information. Ghrelin microinjected into the DVC excited most GD-E and GD-I neurons, which could be completely abolished by pretreatment with [D-Lys-3]-GHRP-6 in the DVC. These results attested that exogenous ghrelin can activate the GD-sensitive neurons in the DVC and this excitatory effect is mediated by its receptor GHSR-1a in the DVC. Otherwise, the excitatory effect could be reduced by cisplatin. Furthermore, cisplatin induced a decreased expression of ghrelin and an increased expression of GHSR-1a (Gong et al., 2017b). Combined the above results, it is concluded that cisplatin might damage the gastrointestinal afferent information due to low ghrelin expression in the DVC.

Normally, the physiological effects of the postprandial gastrointestinal system were simulated by gastric distension. The activation effect of the gastric mechanical receptor can be used as a signal of feeding regulation. Many of the nuclei had a response to mechanical stimulation of the gastrointestinal tract, such as the LHA, the DVC, and the supraoptic nucleus (SON) and so on (Cornejo et al., 2018). Therefore, gastric distension usually was regarded as the body's satiety signal (Sun et al., 2006; Luan et al., 2017). Previous results indicate that ghrelin has multiple regulatory effects on GD neuronal activity in the multiple nuclei in the central nervous system, which might play an important role in the excitability of ghrelin on feeding and gastric motility. In our study, we found that most GD neurons in the DVC were activated by ghrelin; this suggested that DVC ghrelin were involved in the afferent information from the gastrointestinal tract, which might be related to the reward mechanism in feeding. Furthermore, cisplatin weakened this afferent information, which might result in the decrease of gastric motility as we revealed in another manuscript (Gong et al., 2017b).

The LHA, one of the most important nuclei of the hypothalamus, regulates gastric motility and controls feeding. The LHA is the most extensive region of the hypothalamus, which is composed of a number of different nuclei. The LHA can accept the information that comes from the internal and external receptors (Sharpe et al., 2017) and is involved in the regulation of food intake, gastrointestinal motility, cognition, and endocrine function (Jia et al., 2007). These results of biochemical, immunohistochemical and electrophysiological studies have confirmed that the LHA has a very wide range of afferent and efferent connections. The research of anatomy and physiology further confirmed that the LHA has the function of regulating gastrointestinal motility, and it is an important integration center involved in the regulation of appetite and feeding behavior (López-Ferreras et al., 2017). The LHA can accept the gastrointestinal sensory signals from the NTS and regulate the ingestion combined with the efferent impulses from the hippocampus and the limbic system of the amygdala (Hewson et al., 2002).

In our present study, it was found that the electrical stimulation of the LHA could appear to have an effect similar to that of exogenous ghrelin. GD-E neurons and GD-I neurons were excited and the firing frequency increased significantly after electrical stimulation of the LHA. The excitatory responses induced by electrical stimulation of the LHA could be partly blocked by pretreatment with the ghrelin receptor antagonist [D-Lys-3]-GHRP-6. There are lots of different kinds of neurons in the LHA and these neurons have extensive fibrous connections to neurons in other brain regions including the DVC. Nevertheless, there is a disputation on the ghrelin synthesis in the central nervous system. There is compelling evidence that there is no ghrelin in the CNS (Sakata et al., 2009; Furness et al., 2011; Kern et al., 2012; Kern et al., 2014; Cabral et al., 2017; Pustovit et al., 2017). However, the presence of ghrelin in the CNS have been identified by RP-HPLC combined C-RIA (Hosoda et al., 2000; Sato et al., 2005), RP-HPLC combined with N-RIA (Mondal et al., 2005), Elisa (Furness et al., 2011; Ergul Erkec et al., 2018), PCR (Lv et al., 2016), Western Blot (Cui et al., 2014; Huang et al., 2011), and immunohistochemistry (Cui et al., 2014; Lv et al., 2016; Russo et al., 2017a; Russo et al., 2017b). A recent report revealed the presence of GOAT and its ability to acylate non-octanoylated ghrelin in the hippocampus (Murtuza and Isokawa, 2017). The main reason for the above disputation might be related with the specificity of detection methods, ghrelin antibody and individual difference. In our previous study, we revealed that LHA projected ghrelin fibers into the DVC and cisplatin destroyed this projection (Gong et al., 2017a). In the present study, we conducted PCR and WB to assure that ghrelin expressed in the LHA. Therefore, when electrical stimulated, the neurons in the LHA excited and neurotransmitters are released to the other brain areas such as the DVC. Combined with our findings, we hypothesized that ghrelin might be one of neurotransmitters in the pathway from the LHA to the DVC. Therefore, ghrelin receptor antagonist [D-Lys-3]-GHRP-6 pretreatment in the DVC partly blocked the excitatory effect of electrical stimulating on the LHA on the GD neurons. Pustovit et al. reported that the ghrelin receptors of the lumbosacral defecation centers have a physiological role in the control of defecation, but their role is not dependent on ghrelin (Pustovit et al., 2017). Hypothalamic neurons co-express GHSR1a and DRD2 to form GHSR1a:DRD2 heteromers (Kern et al., 2012; Kern et al., 2014). Ghsr+/+ mice treated with a neutral GHSR1a antagonist (thetriazole, JMV2959) are resistant to DRD2 agonist-induced anorexia. We are not certain whether DVC neurons co-express GHSR1a and DRD2. Although [D-Lys-3]-GHRP-6 is not a very specific antagonist, it is true that there is no evidence that it blocks DRD2. In summary, we hypothesized that the electrical stimulation excited ghrelin neurons in the LHA to release ghrelin into the DVC and are involved in the regulation of afferent signals from the gastrointestinal tract. In addition, the projection from the LHA to the DVC was destroyed by cisplatin, which caused the attenuated excitatory effect of electrical stimulating the LHA on the discharge of ghrelin excited GD neurons in the DVC. However, much work remains to be done in the future to interpret the synthesis, physiology, and pharmacology

of ghrelin.

5. Conclusion

In conclusion, our study demonstrated that the exogenous ghrelin in the DVC and the electrical stimulation of the LHA could excite the GD neurons in the DVC. This excitatory effect was mediated by its receptor GHSR-1a and this excitatory effect was attenuated by cisplatin injected intraperritoneally.

6. New findings

6.1. What is the central question of this study?

What is the effect of ghrelin in the dorsal vagal complex and electrical stimulation of the lateral hypothalamus area on the discharge activities of gastric distention neurons in cisplatin-treated rats?

6.2. What is the main finding and its importance?

The discharges of gastric distention sensitive neurons in the dorsal vagal complex could be excited by microinjecting ghrelin into the dorsal vagal complex and electrical stimulation of the lateral hypothalamus area, respectively. The excitatory effect was attenuated by cisplatin intraperitoneally in rats.

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Conflict of Interest

None of the authors has personal or financial conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.03.014.

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