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Nesfatin-1 Influences the Excitability of Gastric Distension-Responsive Neurons in the Ventromedial Hypothalamic Nucleus of Rats

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Summary

The present study investigated the effects of nesfatin-1 on gastric distension (GD)-responsive neurons *via* an interaction with corticotropin-releasing factor (CRF) receptor signaling in the ventromedial hypothalamic nucleus (VMH), and the potential regulation of these effects by hippocampal projections to VMH. Extracellular single-unit discharges were recorded in VHM following administration of nesfatin-1. The projection of nerve fibers and expression of nesfatin-1 were assessed by retrograde tracing and fluoro-immunohistochemical staining, respectively. Results showed that there were GD-responsive neurons in VMH; Nesfatin-1 administration and electrical stimulation of hippocampal CA1 sub-region altered the firing rate of these neurons. These changes could be partially blocked by pretreatment with the non-selective CRF antagonist astressin-B or an antibody to NUCB2/nesfatin-1. Electrolytic lesion of CA1 hippocampus reduced the effects of nesfatin-1 on VMH GD-responsive neuronal activity. These studies suggest that nesfatin-1 plays an important role in GD-responsive neuronal activity through interactions with CRF signaling pathways in VMH. The hippocampus may participate in the modulation of nesfatin-1-mediated effects in VMH.

Key words

Nesfatin-1 • Ventromedial hypothalamic nucleus • Hippocampus • Gastric distension responsive neurons

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Introduction

Nesfatin-1, the 82 amino acid cleavage product from nucleobindin-2 (NUCB2), is a recently discovered regulatory brain-gut peptide (Oh *et al.* 2006). Nesfatin-1 serves various roles in both in the central and peripheral nervous systems. Nesfatin-1 is widely distributed in the central nervous system (CNS) (Goebel-Stengel *et al.* 2011), with expression in several regions, including ventromedial hypothalamic nucleus (VMH) and hippocampus. Physiologically, nesfatin-1 inhibits nocturnal feeding behaviors and gastrointestinal motility in mice (Atsuchi *et al.* 2010) and also inhibits the release of gastric acid (Xia *et al.* 2012). Further, central injection of nesfatin-1 decreases gastric emptying of rats (Stengel *et al.* 2009b). Electrophysiological studies show that nesfatin-1 may exert its anorexic effect role *via* suppression of neuropeptide Y-expressing neurons in the hypothalamic arcuate nucleus (Price *et al.* 2008). Alternatively, corticotropin-releasing factor (CRF) receptor signaling, as well as oxytocin and α-melanocyte-stimulating hormone (α-MSH), are also important for appetite suppression (Tabarin *et al.* 2007) and may be involved in the inhibitory effects of nesfatin-1 on food

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intake and gastric motility.

The hippocampus is an important integrative relay station of the brain limbic system, involved in learning, memory and emotion. Many peptides involved in energy balance have been shown to be expressed in the hippocampus, including nesfatin-1 (Goebel-Stengel *et al.* 2011), CRF (Çalışkan *et al.* 2015) and leptin (Kim *et al.* 2015). Further, the hippocampus has been shown to be involved in digestive function (Chowdhury *et al.* 2014), through the processing of visceroceptive information mainly through reciprocally connections with hypothalamus, amygdala and medulla oblongata (Wenzel *et al.* 1976). The hypothalamus is essential for processing afferent signals from the gut as well as efferent signals that modulate energy balance (Snowball *et al.* 2000). VMH is essential for these processes, serving as the brain's "satiety center", as lesions of VMH causes hyperphagia and obesity (Schwartz *et al.* 2000).

Therefore, in the present study, we examined whether nesfatin-1 regulate the electrical activity of gastric distension (GD)-responsive neurons in VMH, to explore whether nesfatin-1 exerts its influence on these neurons through an interaction with the CRF signaling pathway. Further, we investigated the role for hippocampal input to VMH on GD-responsive neuronal discharges.

Methods

Experimental animals

Adult male Wistar rats (250-300 g, provided by Qingdao Marine Drug Institution, Qingdao, Shandong, China) were used for the experiment. The rats were housed in a regulated environment (22-28 ºC, exposed to lights on from 8:00 a.m. to 8:00 p.m.). Standard laboratory chow pellets and tap water were available *ad libitum*. All animal experiments were approved and performed in accordance with Institutional guidelines of the Animal Care and Use Committee (IACUC) at Qingdao University and IACUC has specifically approved this study.

Retrograde tracing and immunohistochemistry

Rats (n=5) were anesthetized with 10 % chloral hydrate (3 ml/kg, i.p., Sigma, St Louis, MO, USA) and anesthesia was confirmed by the absence of the paw pinch reflex. Each rat was positioned on a stereotaxic apparatus (Narashige SN-3, Tokyo, Japan) with its core temperature maintained at ±37 ºC by a feedback-controlled heating pad. A single pressure injection of 0.1 µl 3 % (w/v) FG (Fluorochrome; Sigma, St. Louis, MO, USA; dissolved in distilled water) was made stereotaxically into the unilateral VMH (relative to bregma: posterior 2.28-2.64 mm, lateral (right) 0.1-1.2 mm and depth 9.0-10.0 mm) at coordinates derived from the atlas of Paxinos and Watson (2007). Seven days after the FG injection, the rats were perfused transaortically with 200 ml of 0.9 % saline, followed by 200 ml 4 % (w/v) paraformaldehyde solution in 0.1 M phosphate buffer (PB; pH 7.4). The brain was removed immediately and postfixed for 4 h in 4 % paraformaldehyde, then cryoprotected in 30 % sucrose solution for 2 days at 4 °C. The brain was cut serially into 15 µm thick frontal sections on a freezing microtome (Kryostat 1720, Leica, Germany).

After the sections were incubated with primary nesfatin-1 (1-82, Rat) antibody (polyclonal; dilution 1 : 300; Phoenix Pharmaceuticals, Burlingame, CA, USA) at 4 °C for 40 h, they were incubated with a fluorochrome-labeled secondary antibody (Cy3-conjugated goat anti-rabbit IgG; dilution 1 : 500; Jackson Immunoresearch, West Grove, PA, USA) for 2 h. The sections were mounted with Citifluor (Citifluor, London, UK). All fluorophores were visualized and photographs were taken under a BX50 microscope and a DP50 digital camera (Olympus, Tokyo, Japan). Rats with inaccurate injection sites were eliminated from the study.

Electrophysiology

The rats (n=114), fasted for 18 h, were anesthetized with 10 % chloral hydrate (0.3 ml per 100 g, i.p.). A small incision was made into the fundus after median laparotomy and the stomach was washed with warm isotonic saline. A latex balloon (3-4 cm in length) attached to polyethylene tubing (PE-240) was inserted into the stomach and fixed on the edge of the incision by a ligature to produce gastric distension by increasing the volume of the balloon (3-5 ml 37 ºC water at a rate of 0.5 ml/s) (Xu *et al.* 2008). Distension was maintained at a constant volume for 10-30 s. The pylorus was ligatured to avoid duodenal reflux and then the abdomen was closed.

In cranial surgery, the dorsal surface of the brain was exposed and the open part was covered with warm agar (3 % in saline) to improve stability for neuronal recording. For micro-pressure injection and extracellular electrophysiological recording, a four-barrel glass microelectrode (total tip diameter 3-10 µm, resistance

5-20 MΩ) was advanced in 10 μm steps with the aid of hydraulic micropositioner into the area of VMH (position described as above). The recording glass microelectrode was filled with 0.5 M sodium acetate and 2 % pontamine sky blue. The other three barrels connected with a four-channel pressure injector (PM2000B, Micro Data Instrument, Inc., USA) were filled with a 10 nM solution of nesfatin-1 (1-82), a 200 nM solution of astressin-B (Sigma, St Louis, MO, USA), a non-selective corticotropin-releasing factor (CRF) antagonist, or anti-NUCB2/nesfatin-1 antibody (Phoenix Pharmaceuticals, Burlingame, CA, USA), and normal saline (NS), respectively. Drugs were ejected onto the surface of firing cells with short-pulse gas pressure (1500 ms, 5.0-15.0 psi) (Trudel *et al.* 2002).

Once the microelectrode was advanced into the VMH, the extracellular action potentials of single neurons were recorded by the glass microelectrode (another electrode was placed on the epicranium of the rat), amplified using a high input impedance amplifier (MEZ8201, Nihon Kohden, Tokyo, Japan), and displayed on an oscilloscope (VC-11, Nihon Kohden, Tokyo, Japan). All signals measured with or without gastric distension were recorded and stored in a computer for further analyses. After a firing pattern was stabilized, the unit was tested with a GD stimulus to determine whether there was input from gastric mechanoreceptors. Units were then classified as gastric distension excitatory (GD-E) neurons if their spontaneous discharge was (transiently) increased in frequency by at least 20 % during gastric distension, or as gastric distension inhibitory (GD-I) neurons when their frequency decreased by at least 20 %.

Electrical stimulation and electrolytic lesion

A monopolar stimulation electrode was advanced into the hippocampal CA1 region (relative to bregma: posterior 3.14-3.6 mm, lateral (right) 1.3-2.0 mm and depth 2.6-3.2 mm) by bipolar concentric electrodes with an interpole distance of 0.5 mm and an external electrode diameter of 100 μm. Stimulation was from a stimulator with a radio-frequency output of square-wave current impulses, 20 μA in intensity and 0.5 ms in duration, delivered for 10 s at 50 Hz. Sham stimulation was conducted as the same procedure with the electrical stimulation but no current was passed through the electrode.

To identify the possible regulation of VMH by hippocampus, electrolytic lesions of the hippocampal CA1 region were performed. In this experiment, 24 rats were randomly divided into 4 groups (6 rats for each group): sham lesion + NS group (SL+ NS), sham lesion + nesfatin-1 group (SL+ nesfatin-1), electrical lesion + NS group (EL + NS), electrical lesion + nesfatin-1 group (EL + nesfatin-1). The electrodes were delivered unilaterally to the CA1 region of the hippocampus by passing 1 mA of anodal direct current for 20 s. Sham lesion was carried out according to the same procedure, without current passed through the electrode. All rats had 5 days recovery from the lesion procedure.

Histological verification

To check the position of the recording electrode, at the end of each experiment, a direct current (10 μA, 20 min) was passed through the electrode to form an iron deposit of Pontamine sky blue. After decapitation, brains were removed and postfixed in formalin for 4 h. Coronal 50-μm sections were cut on a freezing microtome and observed under a microscope. Rats with incorrect probe placements were excluded from analysis.

Statistical analysis

Data are presented as mean ± SD and processed with SPSS 17.0 statistics software (IL, USA). Comparisons were made between groups by either Student's t-test (two groups only) or one-way analysis of variance followed by *post-hoc* Bonferroni's test for comparison among means. $P<0.05$ was considered as statistically significant.

Results

Dose-dependent effects of nesfatin-1 on discharge rate of GD-responsive neurons in VMH

First, we sought to establish a dose-dependent relationship between nesfatin-1 and the discharge rate of GD-responsive neurons in VMH. We tested a series of different concentration of nesfatin-1 (0.5, 1, 5, 10, 50, 100 and 150 nM), and showed that 0.5, 1, 5, 10 and 50 nM nesfatin-1 significantly increased the discharge rate of GD-responsive neurons ($P<0.05$-0.01, Fig. 1A). No significant differences between 50, 100, and 150 nM nesfatin-1 were observed (Fig. 1A). 10 nM nesfatin-1 produced a half-maximal response (pEC50, nesfatin-1). Next, the effect of nesfatin-1 on discharge duration of GD-responsive neurons was observed. We saw that 0.5-10 nM nesfatin-1 produced a dose-dependent increase in the duration of GD-responsive neuronal discharge

(P<0.05~0.01, Fig. 1B). However, when nesfatin-1 concentration was higher than 10 nM, the discharge time of the GD-responsive neurons was not markedly extended beyond the change induced by 10 nM (Fig. 1B). Therefore, 10 nM nesfatin-1 was used for the following experiments.

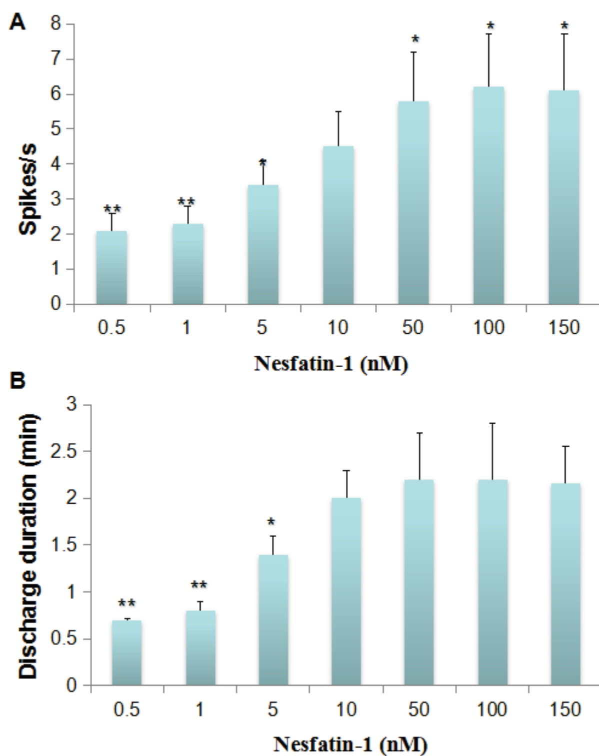

Fig. 1. Dose-dependent effects of nesfatin-1 on VMH GD-responsive neuronal discharge. (**A**) Effect of different concentrations of nesfatin-1 (0.5, 1, 5, 10, 50, 100 and 150 nM) on firing frequency of GD-responsive neurons. *P<0.05, **P<0.01 vs. 10 nM nesfatin-1. (**B**) Effect of different concentrations of nesfatin-1 (0.5, 1, 5, 10, 50, 100 and 150 nM) on the discharge duration of GD-responsive neurons. *P<0.05, **P<0.01 vs. 10 nM nesfatin-1.

Effects of nesfatin-1 on GD-responsive neuronal discharge in VMH

Out of 182 VMH neurons that were recorded from in 54 rats, 124 (68.1 %) responded to gastric distension and thus identified as GD-responsive neurons. Among these, the firing frequency of 65 GD-responsive neurons significantly increased and were classified as GD-E neurons (P<0.05; Figs 2A and 2C); 59 GD-responsive neurons showed a decrease in firing rate and were classified as GD-I neurons (P<0.05; Figs 2B and 2C).

When a GD-responsive neuron was confirmed, nesfatin-1 was microinjected into VMH. Of 65 VMH GD-E neurons examined, 25 (25/65, 38.5 %) showed a decrease in firing frequency in response to nesfatin-1 (P<0.05, Figs 2A and 2C), 18 were activated, and 22 did not respond to nesfatin-1. 20 of 59 (20/59, 33.9 %) GD-I neurons showed a significant increase in the firing frequency compared to saline treatment (P<0.05, Figs 2B and 2C). Further, 16 were inhibited by nesfatin-1, and 23 did not respond. After pretreatment with the CRF antagonist astressin-B, the nesfatin-1-induced responses were partly abolished (P<0.05, Fig. 2). Astressin-B alone had no effect on the activity of the GD-responsive neurons. A control injection of 0.9 % saline was always carried out to confirm the specificity of the responses to nesfatin-1.

FG/nesfatin-1 dual labeled neurons in hippocampus

Next, we performed a retrograde tracing study by injecting FG into VMH. We observed FG-labeled neurons in the hippocampal CA1 region, seven days after injection (Figs 3A and 3D). In the same tissue, nesfatin-1 expression was assessed using a nesfatin-1 (1-82, Rat) antibody. Many nesfatin-1-immunoreactive neurons were observed in CA1 (Figs 3B and 3D), with some nesfatin-1-positive neurons double-labeled with FG (Figs 3C and 3D), arguing that nesfatin-1-expressing neurons in CA1 send projections to VMH.

Effects of hippocampal CA1 electrical stimulation on firing of VMH nesfatin-1/GD-responsive neurons

To observe the physiological relevance of this CA1-VMH connection on GD-responsive neurons, we recorded from VMH GD-responsive neurons during CA1 electrical stimulation. For these experiments, 208 VMH neurons from 60 rats were recorded. 133 (63.9 %) were identified as GD-responsive neurons, out of 70 were GD-E neurons and 63 were GD-I neurons. 26 of 70 (26/70, 37.1 %) GD-E neurons were suppressed by nesfatin-1 and 21 of 63 (21/63, 33.3 %) GD-I neurons showed increased firing rates induced by nesfatin-1. 14 out of 26 (14/26, 53.8 %) GD-E neurons responsive to nesfatin-1 in the VMH were further excited by CA1 electrical stimulation (p<0.01, Figs 4A and 4C), 7 were inhibited and 5 had no response.

Out of 21 GD-I neurons responsive to nesfatin-1, 10 (10/21, 47.6 %) were also excited by CA1 electrical stimulation (p<0.01, Figs 4B and 4C), while 5 were inhibited and 6 had no change. Meanwhile, CA1 electrical stimulation-induced responses were inhibited by pre-treatment with anti-NUCB2/nesfatin-1 antibody to the VMH in either nesfatin-1-responsive GD-E (Figs 4A and

4C, p<0.05) or nesfatin-1-responsive GD-I neurons (Figs 4B and 4C, p<0.05). Injection of anti-NUCB2/nesfatin-1 antibody alone did not change the firing activity of the nesfatin-1-responsive GD-E or GD-I neurons.

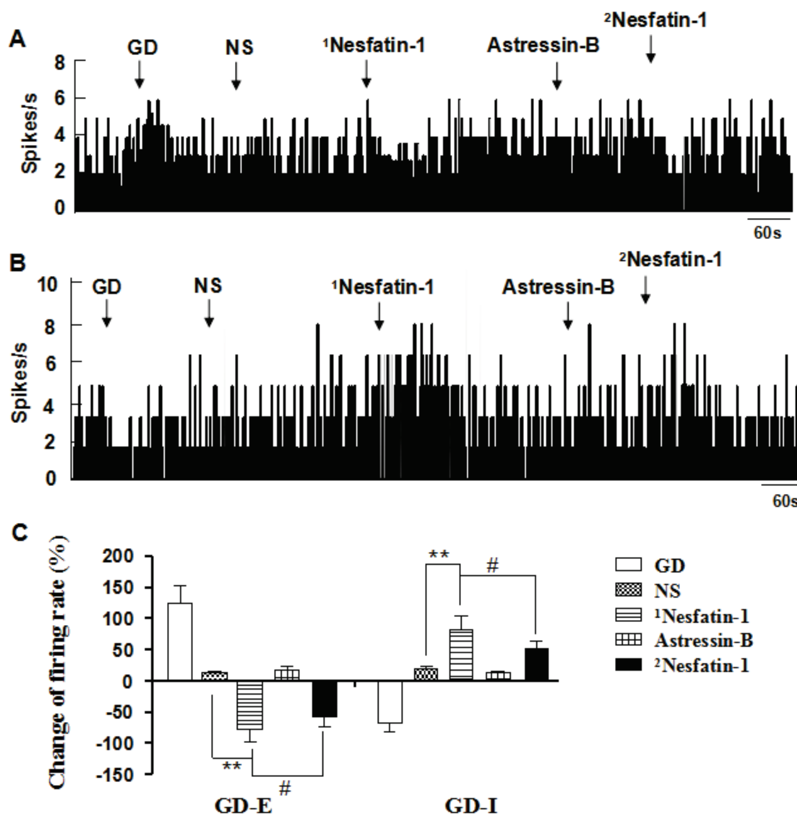

Fig. 2. Effects of nesfatin-1 on the discharge of different types of GD-responsive neurons in VMH. Nesfatin-1 administration to VMH decreased the firing frequency of GD-E neurons (**A**) but increased the firing frequency of GD-I neurons (**B**). After pretreatment with astressin-B, the effects induced by nesfatin-1 were partially diminished. Normal saline or astressin-B alone had no effect on the firing frequency of neurons. (**C**) The change of firing rate (%) of GD-responsive neurons in the VMH induced by nesfatin-1. Scale bar, 60 s. **P<0.01 vs. NS and #P<0.05 vs. ¹nesfatin-1. Data are showed as mean ± SD. The change of firing rate of GD-responsive neurons was calculated by 100 × (firing rate of GD-responsive neurons after treatment − firing rate of GD-responsive neurons before treatment)/(firing rate of GD-responsive neurons before treatment).

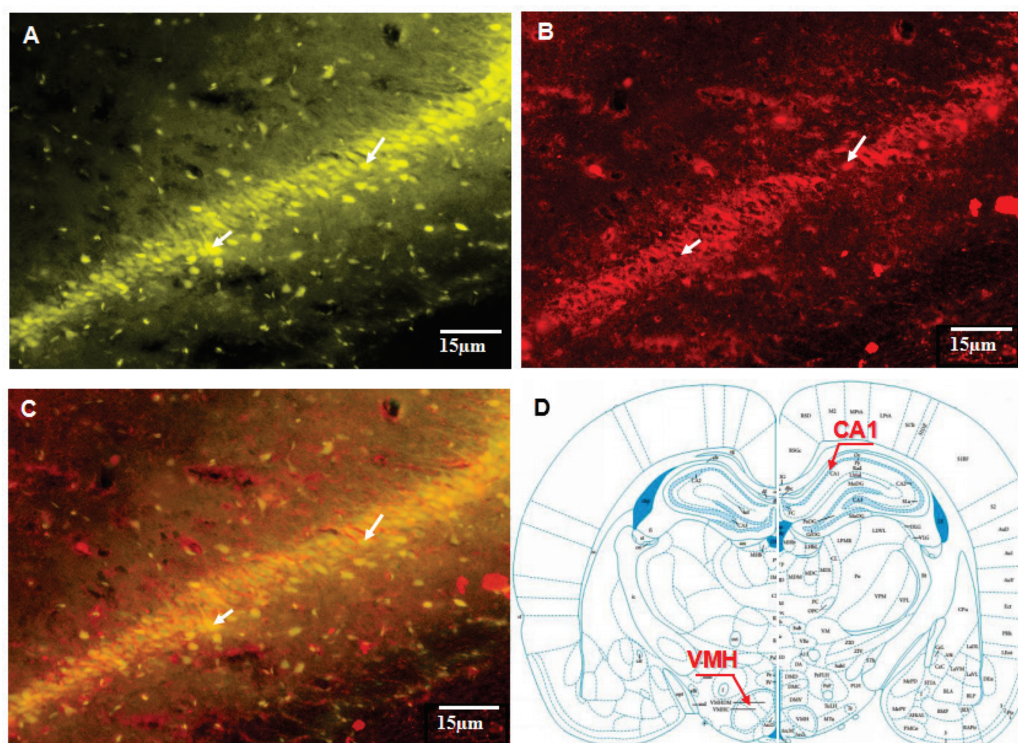

Fig. 3. FG/nesfatin-1 dual labeled neurons in hippocampal CA1 region. Seven days after FG injection in VMH, FG-labelled cells (**A**) and nesfatin-1-positive neurons (**B**) were found in the CA1 hippocampus by immunohistochemical staining of the same tissue. (**C**) Double visualization of FG-labelled cells and nesfatin-1-containing neurons in the hippocampal CA1. (**D**) A schematic diagram for the sites of the hippocampal CA1 region and VMH (Paxinos and Waston 2007). FG, fluorochrome; Scale bars 20 µm (A-C).

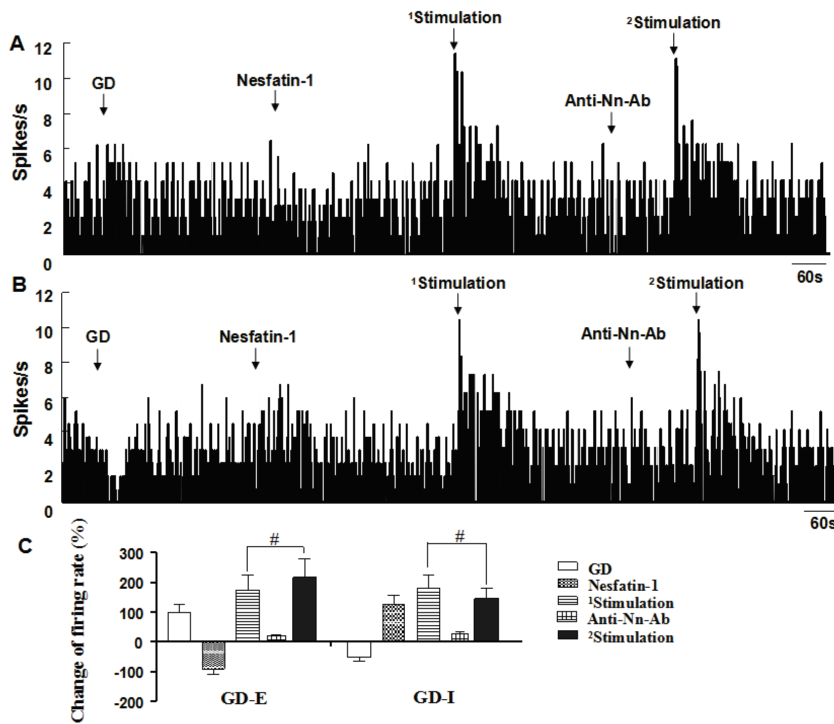

Fig. 4. Effects of CA1 electrical stimulation on dischargeof nesfatin-1/GD-responsive neurons in VMH. Both nesfatin-1-responsive GD-E neurons (**A**) and GD-I neurons (**B**) were excited by electrical stimulation of the hippocampal CA1 region. However, pretreatment with aNUCB2/nesfatin-1 antibody in the VMH partially diminished the increases induced by CA1 stimulation. Scale bar, 60 s. (**C**) The change infiring rate (%) of nesfatin-1-responsive GD neurons in VMH induced by electrical stimulation of the hippocampal CA1 region. #P<0.05 vs. [1]stimulation. Data are showed as mean ± SD. Anti-Nn-Ab, anti-NUCB2/nesfatin-1 antibody.

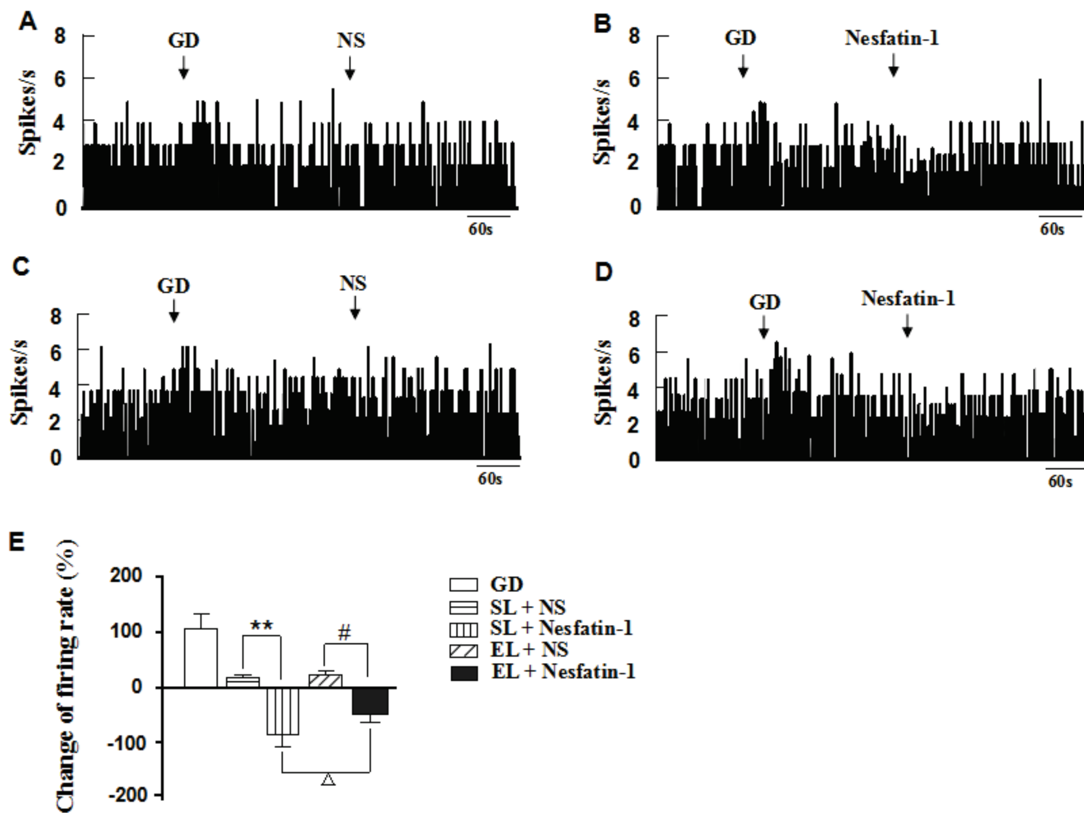

Fig. 5. Effects of elecrolytic lesion of CA1 hippocampus on GD-responsive neuronal firing. (**A**) Sham lesion + NS group (SL+NS); (**B**) Sham lesion + nesfatin-1 group (SL+nesfatin-1); (**C**) Electrical lesion + NS group (EL+NS); (**D**) Electrical lesion + nesfatin-1 group (EL+nesfatin-1). The results showed that the discharge of GD-responsive neurons in VMH did not markedly change after CA1 lesion. However, the firing of GD-responsive neurons was reduced by nesfatin-1 administration to VMH in the hippocampal lesionedrats compared with the sham lesion + nesfatin-1 group (B,D). (**E**) Changes in percent firing rate (%) of GD-responsive neurons in VMH induced by electrical lesion of the hippocampal CA1 region. **P<0.01 vs. SL+NS. #P<0.05 vs. EL+NS. Δ P<0.05 vs. SL+nesfatin-1.

Effects of CA1 electrolytic lesion on VMH GD-responsive neurons

To further characterize CA1 regulation of VMH GD-responsive neuronal discharges, we electrically lesioned CA1 prior to VMH recordings. CA1 lesion, alone, did not affect the firing of VMH GD-responsive neurons. The effect of the nesfatin-1 on VMH GD-responsive neurons were significantly reduced in CA1 lesioned rats compared with sham lesioned rats ($P<0.05$, Fig. 5).

Discussion

The present study demonstrates that nesfatin-1 administration to VMH decreases the firing rate of GD-E neurons and increases firing rate of GD-I neurons *via* an interaction with the CRF signaling pathway. Electrical stimulation of the CA1 region of hippocampus caused excitation of VMH nesfatin-1/GD-responsive neurons. Further, the effect of nesfatin-1 on GD-responsive neurons in the VMH was weakened following electrolytic lesion of CA1. The effect of CA1 electrical stimulation on VMH nesfatin-1/GD-responsive neurons were partially blocked by pretreatment with an anti-NUCB2/ nesfatin-1 antibody in VMH. Furthermore, our retrograde tracing and immunohistochemical staining revealed FG/nesfatin-1 double labeled neurons in CA1 region following FG injection of VMH. Thus, we infer that hippocampal nestfatin-1 neurons modulate the activity of VMH. It follows that nesfatin-1-expressing neurons in the hippocampus project to VMH and are involved in the regulation of gastric motility.

Nesfatin-1 is a novel anorexigenic peptide, cleaved from the larger protein nucleobindin-2 (NUCB2) (Oh *et al.* 2006). Nesfatin-1 displays low expression levels in the hypothalamic paraventricular nucleus after fasting. In rats, the mRNA and protein levels of NUCB2/nesfatin-1 are more prominently expressed in the gastric oxyntic mucosa (Stengel *et al.* 2009a). The effect of nesfatin-1 on gastrointestinal mobility and its role in the regulation of food intake are well known, but the receptor signaling mechanisms and gastrointestinal tract mediating these actions remains unknown. Recent studies demonstrate that nesfatin-1 can cross the blood-brain barrier in both directions in a non-saturable manner (Pan *et al.* 2007). Thus, we investigated whether the administration of nesfatin-1 to the brain affects the regulation of gastrointestinal function. Our study shows that nesfatin-1 regulates GD-responsive neurons in the VMH, neurons that receive afferent signals from the stomach. It is thus implied that central nesfatin-1 may be involved in the regulation of gastric activity.

For many years, VMH has been recognized for its role in processing satiety signals and regulating ingestive behavior and body weight. The VMH has a particularly large number of glucoresponsive neurons, which dynamically respond to hypoglycemia (Routh *et al.* 2003). Recent research has shown that several receptor signaling systems in VMH, including dopamine (Huang *et al.* 2005), GABA (Dellovade *et al.* 2001), histamine (Magrani *et al.* 2004) impact feeding behavior. In our study, we found neurons sensitive to gastric distension sensitive in VMH. We infer that VMH may have a role in the modulation gastric motility. Anatomically, VMH makes connections with many brain regions that are associated with feeding behavior. We used gastric distension as a stimulus of visceral afferent signals because it is a relatively specific and homogeneous class of vagal afferent signal related to food ingestion (Tache *et al.* 2008). Our data indicate that VMH receives this signal from the gastrointestinal tract. We show that administration of exogenous nesfatin-1 to VMH decreases the activity of GD-E neurons, but increase the activity of GD-I neurons. The anorexigenic action of nesfatin-1 may be related to these changes in the activity of GD-responsive neurons. This point can be confirmed by future experiments.

NUCB2/nesfatin-1-immunoreactive neurons co-express several other peptides and neurotransmitters, which are known to be involved with the regulation of food intake, namely corticotropin-releasing factor (CRF) (Foo *et al.* 2008), α-melanocyte-stimulating hormone (α-MSH) (Fort *et al.* 2008), ghrelin (Kerbel and Unniappan 2008), as well as many others. In particular, brain CRF is involved in the stress-induced suppression of gastric motility (Stengel and Taché 2009). Further, circulating levels of both corticosterone and adrenocorticotropin (ACTH) are elevated following intracerebroventricular injection (ICV) administration of nesfatin-1, both of which are essential for hypothalamic-pituitary-adrenal axis function (Konczol *et al.* 2010). Furthermore, bilateral adrenalectomy increases the expression of NUCB2/nesfatin-1 mRNA in the paraventricular hypothalamic nucleus (PVN) (Shimizu *et al.* 2009). CRF receptor related peptides are involved in numerous physiological and behavioral functions, including gastrointestinal regulation. They are also capable of strong

anorectic and thermogenic effects. In fact, the CRF system, which promotes a negative energy profile upon activation, could represent a potential target for the pharmacological treatment of obesity. CRF receptor-expressing neurons in VMH seem to be involved in the regulation of energy intake, maintaining homeostatic steady state (Doyon *et al.* 2004). Injection of CRF receptor antagonists counteracts the anorexigenic function of nesfatin-1 (Gotoh *et al.* 2013). Stimulation of the CRF receptor in the VMH decreases feeding. Further, a negative metabolic state or hyperphagia are associated with decreased expression of CRF receptors in the VMH (Hashimoto *et al.* 2004, Makino *et al.* 1998). In the present study, we have shown that the effects of nesfatin-1 on GD-sensitive neurons could be partially abolished by pretreatment with the CRF non-selective antagonist astressin-B in the VMH. Thus, we hypothesize that nesfatin-1 exerts its anorexigenic effect *via* the CRF receptor signaling pathway in the VMH. This functional relationship between nesfatin-1 and the CRF signaling system may have implications in the understanding of gastrointestinal function disorders and obesity.

In recent years, the hippocampus has received much attention in relation to its potential involvement in feeding and energy regulation (Wang *et al.* 2006). This focus is partly based on the fact that many receptors for neurohormonal signaling molecules, including ghrelin, cholecystokinin, leptin, insulin, are expressed in hippocampus (Davidson *et al.* 2009). Anatomically, direct neural projections from the ventral pole of the hippocampal CA1 to hypothalamic loci known to participate in the control of food intake have been identified (Cenquizca *et al.* 2006). Also, hippocampal lesion has been shown to alter feeding behavior in rats (Clifton *et al.* 1998). Our study found that VMH GD-responsive neuronal discharges were enhanced by administration of nesfatin-1 following electrical stimulation of CA1. This result suggests that the hippocampus is able to regulate the activity of VMH neurons. The retrograde tracing and fluoro-immunohistochemical staining studies confirmed the nesfatin-1-expressing neurons make connections from CA1 to VMH.

Taking together, our data suggest that nesfatin-1 release in VMH modulates GD-responsive neurons, perhaps *via* CRF signaling pathways. Further, the hippocampus participates in this process. These studies provide insight into the role of VMH in the control of gastrointestinal function mediated *via* nesfatin-1.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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