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Nesfatin-1 regulates the lateral hypothalamic area melanin-concentrating hormone-responsive gastric distension-sensitive neurons and gastric function via arcuate nucleus innervation

Luo Xu[a,*], *Hongbo Wang*[b], *Yanling Gong*[c], *Mingjie Pang*[d], *Xiangrong Sun*[a], *Feifei Guo*[a], *Shengli Gao*[a]

[a] *Department of Pathophysiology, Medical College of Qingdao University, Qingdao, Shandong, China*
[b] *Department of Gastroenterology, Jimo People's Hospital, Qingdao, Shandong, China*
[c] *Department of Pharmacy, College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao, Shandong, China*
[d] *Department of Otolaryngology, Qingdao Municipal Hospital (Group), Qingdao, Shandong, China*

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ABSTRACT

Nesfatin-1, a recently discovered neuropeptide involved in satiety. Recent studies have revealed that central nesfatin-1 inhibits gastric emptying and gastric acid secretion, though the mechanisms involved in these processes are not known. We aim to explore the effects of nesfatin-1 on a population of gastric distension (GD)-sensitive neurons in the lateral hypothalamus (LHA), gastric motility, and gastric secretion and the role for an arcuate nucleus (Arc)-LHA neural pathway in these processes. Single unit extracellular discharge recordings were made in of LHA. Further, gastric motility and gastric secretion in rats were monitored. Retrograde tracing and fluorescent immunohistochemical staining were used to explore nesfatin-1 neuron projection. The results revealed that administration of nesfatin-1 to the LHA or electric stimulation of the Arc could alter the neuronal activity of melanin-concentrating hormone (MCH)-responsive, GD-responsive neurons in LHA, which could be blocked by pretreatment with MCH receptor-1 antagonist PMC-3881-PI or weakened by pretreatment of a nesfatin-1 antibody in LHA. Administration of nesfatin-1 into LHA could inhibit gastric motility and gastric secretion, and these effects could be enhanced by administration of PMC-3881-PI. Electrical stimulation of Arc promoted the gastric motility and gastric secretion. Nesfatin-1 antibody or PMC-3881-PI pretreatment to LHA had no effect on Arc stimulation-induced gastric motility, but these pretreatments did alter Arc stimulation-induced effects on gastric secretion. Our findings suggest that nesfatin-1 signaling in LHA participates in the regulation of efferent information from the gastrointestinal tract and gastric secretion which also involve MCH signaling. Further, they show that a nesfatin-1-positive Arc to LHA pathway is critical for these effects.

Abbreviations: GD, gastric distension; LHA, lateral hypothalamus; Arc, arcuate nucleus; MCH, melanin-concentrating hormone; MI, motor index; FG, fluorogold; DMN, dorsomedial nucleus; VMN, ventromedial hypothalamic nucleus.

* Corresponding author. Tel.: +86 13869887501; fax: +86 532 83813986.
E-mail address: xu.luo@163.com (L. Xu).

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1. Introduction

Nesfatin-1 is an anorexigenic peptide derived from the precursor NEFA/nucleobindin 2 (NUCB2) [1]. It is widely expressed in neurons of the forebrain, hindbrain, brainstem and spinal cord, especially those involved in feeding regulation and energy homeostasis [1–6]. Moreover, nesfatin-1 is also distributed in peripheral tissues, such as stomach and adipose tissue [1]. Studies have revealed that nesfatin-1 can inhibit food intake in rodents fed ad libitum [7–9]. Nesfatin-1 also participates in the regulation of gastric motility [10,11]. When delivered i.c.v. to fed animals, nesfatin-1 caused sequence changes in percentage motor index (MI), where MI was decreased in the antrum, whereas the time between the initiation of phase III-like contractions after administration was prolonged in the duodenum. The detailed mechanisms for nesfatin-1 prolonging gastroduodenal motility remain unknown.

The peptide melanin-concentrating hormone (MCH) was first isolated from the salmon pituitary and characterized as an antagonist of alpha-melanocyte-stimulating hormone (α-MSH)-induced skin darkening [6]. Now, MCH is associated with a wide variety of brain functions such as food intake, energy homeostasis, stress response, anxiety, sleep/wake cycle, memory, and reward [12–16]. Little, however, is known about the effects of MCH on the processing of gastric information and gastric motility.

The lateral hypothalamic area (LHA) is an important brain region that regulates multiple processes including feeding, reward behaviors, and autonomic function [17]. The neuropeptides orexin and MCH are found in different neuronal populations in LHA where these neurons innervate several brain regions, regulating various physiological functions including feeding, energy homeostasis, reward behaviors [18]. The arcuate nucleus (Arc) is crucial for the maintenance of energy homeostasis as an integrator of long- and short-term hunger and satiety signals and is considered a critical control center for food intake. As described above, nesfatin-1 is neuronally expressed in both LHA and Arc. However, a synaptic connection between the LHA and the Arc by nesfatin-1-containing neurons and the effects of nesfatin-1 on the gastric motility have not been shown. Therefore, in the present study, we investigate a potential nesfatin-1-immunoreactive projection from Arc to LHA, and further explore whether nesfatin-1 in LHA could influence the firing activity of MCH-responsive, gastric distension (GD)-responsive neurons, nesfatin-1's effects on gastric motility and secretion, and the role for Arc regulation of these measures.

2. Experimental Procedures

2.1. Animals

Male Wistar rats weighing 250–300 g (Qingdao Marine Drug Institution, Shandong, China) were used. They were housed in a temperature-controlled room at 25 ± 2 °C and exposed to light from 08:00 h to 20:00 h. Standard laboratory chow pellets and tap water were available ad libitum. All animal experiments were carried out in accordance with the ethical guidelines of Qingdao University for animal care.

2.2. Chemicals

Rat nesfatin-1 and anti-NUCB2/nesfatin-1 antibody were purchased from Phoenix Pharmaceuticals (CA). Rat MCH and PMC-3881-PI were purchased from Peptides International (Louisville, KY). Thiobutabarbitol, fluorogold (FG) and chloral hydrate were purchased from Sigma-Aldrich Chemical (St. Louis, MO). Cy3-conjugated goat anti-rabbit IgG was purchased from Jackson Immunoresearch (PA) and citifluor was purchased from Citifluor (London, UK). All other general reagents were commercially available.

2.3. Retrograde Tracing and Immunohistochemistry

The rats were anesthetized with chloral hydrate and mounted on a stereotaxic apparatus (Narashige SN-3, Tokyo, Japan). A single of 0.2 μl 3% (w/v) FG (Fluorochrome, Sigma, St. Louis, MO; dissolved in distilled water) was pressure injected into the LHA (bregma: P: 1.3–2.3 mm, L (R): 1.5–2.5 mm, H: 8.0–9.0 mm) [19]. Seven days later, the rats were perfused transaortically with 100 ml of saline, followed by 300 ml of 4% (w/v) paraformaldehyde. The brains were removed immediately and post-fixed for 2 h in 4% paraformaldehyde then cryoprotected in 30% sucrose for 2 days at 4 °C. Each brain was cut serially into 15-μm-thick frontal sections on a freezing microtome (Kryostat 1720, Leica, Germany).

The sections were incubated with primary anti-NUCB2/nesfatin-1 antibody (polyclonal, dilution: 1:400) for 40 h at 4 °C, and then incubated with fluorochrome-labeled secondary antibody (Cy^3-conjugated goat anti-rabbit IgG, dilution: 1:500) for 2 h. The sections were mounted with Citifluor. All fluorophores were visualized and photographs were taken under a BX50 microscope and a DP50 digital camera (Olympus, Tokyo, Japan).

2.4. Surgery and Electrophysiological Recordings

After fasting overnight, the rats were anesthetized with thiobutabarbitol. The process of balloon implantation in stomach and cranial surgery was carried out as described previously [20].

After the abdominal surgery, the rat was positioned on the stereotaxic frame and a small hole was drilled in the skull to expose the cortex and the dura was cut. A five-barrel glass microelectrode was advanced in 10-μm steps to the region of the LHA and used for single neuron recording and micropressure injection. The recording glass microelectrode was filled with 0.5 M sodium acetate and 2% Pontamine sky blue. The other four barrels connected with a four-channel pressure injector (PM2000B, Micro Data Instrument, NJ) and filled with 200 nM solution of MCH, 10 nM solution of nesfatin-1, 800 nM solution of PMC-3881-PI (an antagonist of MCH receptor-1), and normal saline respectively. Drugs were ejected on the surface of firing cells with short pulse gas pressure (1500 ms, 5.0–15.0 psi) [21].

Once the microelectrode was advanced into the LHA, the extracellular action potentials of single neurons were recorded by the glass microelectrode, amplified using a high input impedance amplifier (MEZ8201, Nihon Kohden, Tokyo, Japan), and displayed on an oscilloscope (VC-11, Nihon Kohden,