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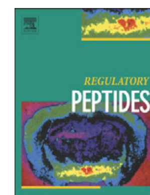

Nesfatin-1 influences the excitability of glucosensing neurons in the hypothalamic nuclei and inhibits the food intake

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ABSTRACT

Nesfatin-1 is a recently discovered neuropeptide that has been shown to decrease food intake after lateral, third, or fourth brain ventricle, cisterna magna administration, or PVN injection in ad libitum fed rats. With regards to the understanding of nesfatin-1 brain sites of action, additional microinjection studies will be necessary to define specific nuclei, in addition to the PVN, responsive to nesfatin-1 to get insight into the differential effects on food intake. In the present study, we evaluated nesfatin-1 action to modulate food intake response upon injection into the specific hypothalamic nuclei (PVN, LHA and VMN) in freely fed rats during the dark phase. We extend previous observations by showing that the nesfatin-1 (50 pmol) injected before the onset of the dark period significantly reduced the 1 to 5 h cumulative food intake in rats cannulated into the PVN, LHA, but not in rats cannulated into the VMN.

Glucosensing neurons located in the hypothalamus are involved in glucoprivic feeding and homeostatic control of blood glucose. In order to shed light on the mechanisms by which nesfatin-1 exerts its satiety-promoting actions, we examined the effect of nesfatin-1 on the excitability of hypothalamic glucosensing neurons. Nesfatin-1 excited most of the glucose-inhibited (GI) neurons and inhibited most of the glucose-excited (GE) neurons in the PVN. Of 34 GI neurons in the LHA tested, inhibitory effects were seen in 70.6% (24/34) of GI neurons. The main effects were excitatory after intra-VMN administration of nesfatin-1 in GE neurons (27/35, 77.1%). Thus, our data clearly demonstrate that nesfatin-1 may exert at least a part of its physiological actions on the control of food intake as a direct result of its role in modulating the excitability of glucosensing neurons in the PVN, LHA and VMN.

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1. Introduction

Nesfatin-1, derived from the precursor NEFA/nucleobindin 2 (NUCB2), was recently identified as anorexigenic signal, acting in a leptin-independent manner [1]. NUCB2 mRNA expression was detected in rat hypothalamic and brainstem nuclei implicated in the regulation of food intake, notably in the arcuate nucleus, paraventricular nucleus (PVN), supraoptic nucleus, lateral hypothalamic area (LHA), zona incerta and the nucleus of the solitary tract (NTS) [1–4]. Nesfatin-1 has been shown to inhibit food intake after lateral (icv) [5,6], third (3v) [1,7], or fourth brain ventricle (4v) [5], cisterna magna administration [5], or PVN injection [7] in ad libitum fed rats, and in mice when injected into the 3v [7]. As Stengel et al. [5] pointed out that with regards to the understanding action site of nesfatin-1 in brain, additional microinjection studies will be necessary to define specific nuclei, in addition to the PVN, responsive to nesfatin-1 to get insight into the differential effects on food intake and digestive functions. Hypothalamic areas including the PVN, LHA and VMN are important centers in the control of food intake [8]. Nesfatin-1 is produced and influences the membrane potential of neurons in the PVN [1,9], an area of the brain that is considered an integrative center for regulation of feeding. The LHA contains neurons expressing melanin-concentrating hormone (MCH, glucose-excited, i.e. GE neurons) and orexin neurons (glucose-inhibited, i.e. GI neurons). The VMN plays an important role in the mechanism by which the brain detects a falling glucose and triggers a counterregulatory hormone response [8].

Neural mechanisms involved in nesfatin-1's anorexigenic effect encompass the recruitment of several hypothalamic and medullary anorexigenic pathways [1,5,7]. The recently established crosstalk between nesfatin-1 and corticotropin-releasing factor2 (CRF), oxytocin and melanocortin pathways involved in hypothalamic nesfatin-1's anorexigenic action are still to be further clarified. To investigate which nuclei are involved in the feeding response, we measured food intake in response to microinjection of nesfatin-1 into defined hypothalamic sites.

Abbreviations: LHA, lateral hypothalamic area; PVN, paraventricular nucleus; VMN, ventromedial hypothalamic nucleus.

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In vivo studies have suggested that glucosensing neurons located in the hypothalamus and lower brainstem are involved in glucoprivic feeding and homeostatic control of blood glucose [10,11]. It is only recently that they have received more attention due to their potential role in the regulation of appetite [8]. Hypothalamic glucose-excited (GE) neurons increase their electrical activity in response to elevated glucose levels, whereas glucose-inhibited (GI) neurons decrease their activity during hyperglycaemia [8].

In order to shed light on the mechanisms by which nesfatin-1 exerts its satiety-promoting actions, this study examined the effect of nesfatin-1 on the excitability of hypothalamic glucosensing neurons. In addition, we evaluated nesfatin-1 action to modulate food intake response upon injection into the specific hypothalamic nuclei (LHA, PVN and VMN) in freely fed rats during the dark phase.

2. Materials and methods

2.1. Animals

To examine the effect of nesfatin-1 on food intake, adult male Wistar rats (Qingdao Institute for Drug Control) weighing 280–330 g were housed under controlled illumination (08:00–20:00) and temperature (22 ± 2 °C). Rats had free access to standard rodent chow (Jinan Kangda Lab Diet) and tap water. Protocols have been approved by the Qingdao University Animal Care and Use Committee.

2.2. Food and water intake

Rats were anesthetized with chloral hydrate (80 mg/ml, 0.5 ml/100 g, i.p.). Anesthetized animals were positioned in a stereotaxic apparatus (Narishige SN-3, Tokyo, Japan) with the incisor bar 3.3 mm below the center of ear bars, and the skull was exposed. A stainless steel cannula (26 gauge, 15 mm) was implanted into the hypothalamic nuclei with the aid of a stereotaxic device. Stereotaxic coordinates were obtained from Paxinos and Watson's brain atlas [12]: PVN (1.8 mm caudal to the bregma, 0.5 mm lateral to the middle line, and 7.0 mm below the surface of the skull); LHA (3.0 mm caudal to the bregma, 2.0 mm lateral to the middle line and 7.5 mm below the surface of the skull); VMN (2.5 mm caudal to the bregma, 0.5 mm lateral to the middle line and 8.3 mm below the surface of the skull). After anchoring the cannula and sealing all skull openings with dental acrylic, the incision was sutured, and a 28-gauge obturator was placed in the cannula. After 7 day recovery period, rats were handled for another at least 3 days in metabolic cages (Feeding and Activity Analyser 47552-002, Ugo Basile, Italy) with free access to lab chow and tap water and became accustomed to the injection procedure.

On the day of experiments, food was removed from cages at 15:00. Rats received an injection of nesfatin-1 (1-82, Phoenix Pharmaceuticals, Burlingame, CA, 50 pmol in 0.5 μl of volume) at 19:30. Animals were administered nesfatin-1 with a 28-G injector via a microsyringe which extended 0.7 mm below the guide cannula, and control group was injected with equal volume of saline. Nesfatin-1 injection (50 pmol) into the PVN significantly decreased cumulative food intake at 1–4 h after injection [7]. This dose, therefore, was used to compare the potency of the anorexigenic action of nesfatin-1 among each of the hypothalamic nuclei, which was the primary aim of this study. For all experiments, nesfatin-1 or saline was delivered to the nuclei parenchyma over a 2 min period and the injector was kept in place for an additional 5 min. Then food was returned to cages, and cumulative food intake for the following 1–12 h was measured by measuring the weight of the food containers with electronic precision scales. The outputs were continuously monitored by Data Acquisition software 51800 (Feed-Drink Monitoring System Ver. 1.31, Ugo Basile, Italy). After all experiments were completed, brains were removed and stored in a 4% paraformaldehyde solution; the injection sites were stained with pontamine sky blue and sectioned in the coronal plane on a freezing microtome (Kryostat 1720, Leica, Germany) at a thickness of 50 μm to verify the location of the cannula. Only data for rats whose injection site was located in right placements were included in the study. Rats with misplaced cannulas were excluded retrospectively.

2.3. Electrophysiological recordings

The rats were anesthetized with urethane (1.0 g/kg, i.p.) and a maintenance dose of anesthetics was given whenever necessary. Anesthetized animals were placed in a stereotaxic apparatus (Narishige SN-3, Tokyo, Japan) with the incisor bar 3.3 mm below the center of ear bars. Stereotaxic coordinates [12] were as follows: PVN (1.6–1.9 mm caudal and 0.1–0.7 mm lateral to the bregma, 7.7–8.4 mm below the surface of the skull); LHA (1.8–3.2 mm caudal to the bregma, 1.5–2.5 mm lateral to the sagittal sinus, and 7.5–9.0 mm below the surface of the skull); VMN (1.8–3.1 mm caudal to the bregma, 0.2–1.0 mm lateral to the sagittal sinus, and 9.3–10.0 mm below the surface of the skull). Rectal temperature was maintained at 36–38 °C. Removing a portion of the skull and dura mater, the dorsal surface of the brain was exposed, then covered with warm agar (3–4% in saline) to improve stability for neuronal recording.

Four-barrel glass microelectrode (total tip diameter 3–10 μm, resistance 5–20 MΩ) was used for electrophysiological recording and micro-pressure injection as previously described [13]. The recording glass microelectrode was filled with 0.5 M sodium acetate and 2% pontamine sky blue. The other three barrels connected with 4 channel pressure injector (PM2000B, Micro Data Instrument, Inc. USA) were filled with 5 mM solution of glucose (PH 7.4) [14], 1.5×10^{-8} M solution of nesfatin-1, and 0.9% NaCl, respectively. Drugs were ejected on the surface of firing cells with short pulse gas pressure (1500 ms, 10.0 psi) [13]. The intrabarrel drug concentrations were chosen on the basis of their efficacy to reliably alter cell firing. Less than 1 nl of nesfatin-1 was applied to the firing cells during extracellular recording. The recorded electrical signals were amplified by a microelectrode amplifier (MEZ-8201, Nihon Kohden) and passed through low and high pass filters between 0.3 and 3 kHz. Electrical signals were displayed on a memory oscilloscope (VC-11, Nihon Kohden) and monitored on an audiomonitor. The amplified electrical signals were sent through an A/D interface (Power 1404, CED, United Kingdom) into a laboratory computer, which was used to analyze the data online. Spike times were preprocessed online and further analyzed offline using the program of Histogram ver.1.00 (Shanghai University) for spike data analysis. A change at least 20% of basal firing rate during drug application was considered significant. Drug application was performed only once for each recording and a period of 30 min was allowed to pass before another recording in the same track. At least 3 min of baseline firing was collected from each cell before drug injection into the hypothalamic nuclei. The frequency of control was determined by the average frequency of 120 s baseline data before drug application. The maximal change of frequency within 50 s following drug application was calculated as drug effect.

To verify the correct placement 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. The rats were perfused transcardially with 0.9% saline, followed by 10% buffered Formalin solution. The brains were removed, 50 μm frozen coronal sections were cut through the regions of the hypothalamus, stained with neutral red, cleared with xyline, and coverslipped. All the recording and microinjection sites were verified under light microscope.

2.4. Statistical analysis

Data were expressed as means ± standard error of the mean (S.E.M.). Paired *t*-test was used to compare the difference of firing

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