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Research Report

Effects of ghrelin on hypothalamic glucose responding neurons in rats

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Abstract

Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) with potent stimulatory effects on food intake. The aim of the present study was to investigate the effects of ghrelin on neuronal activity of hypothalamic glucose responding neurons. Single unit discharges in the lateral hypothalamic area (LHA), the ventromedial hypothalamic nucleus (VMH), and the parvocellular part of the paraventricular nucleus(pPVN) were recorded extracellularly by means of four-barrel glass micropipettes in anesthetized rats. The activity of glucose-sensitive neurons (GSNs) in the LHA, pPVN, and of glucoreceptor neurons (GRNs) in the VMH modulated by administration of ghrelin was analyzed. In the LHA, the majority of GSNs (17/25) increased in frequency due to ghrelin. Whereas the majority of VMH-GRNs (27/33) and pPVN-GSNs (9/13) was inhibited. The responses to ghrelin were abolished by pretreatment of [D-Lys-3]-GHRP-6, ghrelin receptor antagonist. These data indicate that the glucose responding neurons in the LHA, VMH, and pPVN are also involved in the orexigenic actions of ghrelin in the hypothalamic circuits, although AgRP/NPY neurons in the arcuate nucleus (ARC) are the primary targets of ghrelin.

Theme: Neural basis of behavior
Topic: Central control of ingestion

Keywords: Ghrelin; Glucose-sensitive neuron; Glucoreceptor neuron; LHA; VMH; pPVN

1. Introduction

Ghrelin is a recently identified endogenous ligand of the growth hormone secretagogue receptor (GHS-R) [15]. It was originally isolated from the stomach [5,6], but has also shown to be present in the rat hypothalamus [4,18]. Recent data have led to the recognition that ghrelin plays an important role in body-weight regulation and energy homeostasis because its administration increases food intake and causes fat and weight gain in rodents [23,31]; the orexigenic effect of ghrelin seems to be independent of its GH-releasing activity [14]. It has been found that circulating levels of ghrelin increase following a 48-h fast, and infusion of glucose into the stomach decreases plasma ghrelin concentration [22,31].

Information accumulated over the past decade has revised our views on the hypothalamic control of appetite. Hypothalamic areas including the paraventricular nucleus (PVN), perifornical area (PFA), and the lateral hypothalamic area (LHA) are richly supplied by axons from the arcuate nucleus (ARC) NPY/AgRP and POMC/CART neurons [8,29]. The recent studies have shown that injection of ghrelin into the cerebrospinal fluid (CSF) induces c-*fos* expression in the PVN, dorsomedial (DMH), VMH, and

Abbreviations: AgRP, agouti-related peptide; CART, cocaine- and amphetamine-regulated transcript; POMC, proopiomelanocortin; GSN, glucose-sensitive neuron; GRN, glucoreceptor neuron; ARC, arcuate nucleus; LHA, lateral hypothalamic area; pPVN, parvocellular part of paraventricular nucleus; VMH, ventromedial hypothalamic nucleus; GHS-R, growth hormone secretagogue receptor

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ARC of the hypothalamus, as well as in the nucleus of the solitary tract (NTS) and area postrema (AP) of the brain stem [17]. It has been established that the glucose-sensitive neurons (GSNs) in the LHA, pPVN, and glucoreceptor neurons (GRNs) in the VMH are involved in the control of food intake [25]. Furthermore, it has been shown that the activity of GSNs in the LHA was suppressed by leptin, whereas the activity of GRNs in the VMH was facilitated; in contrast, orexin-A had opposite effects [30]. The present study was undertaken to examine the effects of ghrelin on the glucose responding neurons in the LHA, VMH, and pPVN.

2. Materials and methods

2.1. Animals

Adult Wistar rats (Qingdao Institute for Drug Control) of either sex, weighing 220–280 g, were used. They were housed under conditions of controlled illumination (12:12-h light/dark cycle, lights on/off: 8:00 a.m./8:00 p.m.), humidity, and temperature (22 ± 2 °C) for at least 7 days prior to the experiments. Standard laboratory chow pellets and tap water were available ad libitum. All animal experiments were carried out in accordance with the ethic guidelines of Qingdao University for animal care.

2.2. Electrophysiological recordings

Rats were anesthetized with urethane (1.0 g/kg, i.p.) and a maintenance dose of anesthetics was given whenever necessary. 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, the dorsal surface of the brain was exposed. Stereotaxic coordinates were as follows: LHA (1.8–2.3 mm posterior to the bregma, 1.5–2.5 mm lateral to the sagittal sinus, 7.5–9.0 mm ventral from the dura); VMH [P: 2.8–3.3 mm, L(R): 0.2–1.0 mm, H: 9.3–10 mm]; pPVN [P: 1.8–2.3 mm, L(R): 0.1–0.4 mm, H: 7.7–8.4 mm] [26]. Rectal temperature was maintained at 36–38 °C.

Four-barrel glass microelectrode (total tip diameter 3–10 μm, resistance 5–20 MΩ) was used for electrophysiological recording and micro-pressure injection. 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 2 M solution of glucose (pH 7.4), 15 nM solution of ghrelin, and 28 nM solution of [D-Lys-3]-GHRP-6 (each was dissolved in 0.9% NaCl) and 0.5 M NaCl, respectively. The barrel filled with 0.5 M NaCl was used to rule out the osmotic effects and any neurons that responded to Na^+ or Cl^- applications were omitted from the results.

Rat ghrelin and the ghrelin receptor antagonist ([D-Lys-3]-GHRP-6) were generously supplied by Dr. T.L. Peeters (Gut Hormone Laboratory, Leuven, Belgium). Drugs were ejected on the surface of firing cells with short pulse gas pressure (1500 ms, 5.0–15.0 psi) [13]. The intrabarrel drug concentrations were chosen on the basis of their efficacy to reliably alter cell firing. Volumes less than 1 nl of ghrelin were applied to the firing cells during extracellular recording.

The recorded electrical signals were amplified and displayed on a Memory Oscilloscope (VC-11, Nihon Kohden), the analog signals were fed into a signal analyzer and computer which incorporated a signal discriminator to allow unitary data to be stored on-line.

2.3. 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. 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.

2.4. Data analysis

Data were expressed as means ± standard error of the mean (SEM). Comparisons of agents induced responses before (pre-) and after (post-) treatment were made by Student's t test; the differences of the percentages between GSNs and non-GSNs responding to ghrelin or [D-Lys-3]-GHRP-6 or not responding on LHA, VMH, and pPVN neurons were tested by means of the χ^2 test. Differences were considered to be significant at $P < 0.05$.

3. Results

Results of ghrelin on hypothalamic GSNs and non-GSNs are summarized in Table 1. 25 (35%) GSNs in 72 LHA neurons were identified by their suppression in response to applied glucose. Of 25 LHA-GSNs tested with ghrelin, 17 (68%) GSNs were excited. 33 (40%) GRNs in 81 VMH neurons were identified by their facilitation in response to

Table 1
Effects of ghrelin on hypothalamic neurons

	Decrease	Increase	No effect
LHA			
25 GSNs	3	17	5
47 Non-GSNs	8	2	37
VMH			
33 GRNs	27	2	4
48 Non-GRNs	5	19	24
pPVN			
13 GSNs	9	1	3
36 Non-GSNs	5	9	22