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Effects of ghrelin on glucose-sensing and gastric distension sensitive neurons in rat dorsal vagal complex

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Abstract

Ghrelin has been identified as the endogenous ligand of the growth hormone secretagogue receptor (GHS-R). Recent studies have shown that site-specific injection of ghrelin directly into the dorsal vagal complex (DVC) of rats is equally as sensitive in its orexigenic response to ghrelin as the arcuate nucleus of the hypothalamus (ARC). It is as yet unclear how circulating ghrelin would gain access to and influence the activity of the neurons in the DVC in which GHS receptors are expressed. In the present study, neuronal activity was recorded extracellularly in the DVC of anesthetized rats in order to examine the effects of ghrelin on the glucosensing neurons and the gastric distension (GD) sensitive neurons. The 82 neurons were tested with glucose, of which 26 were depressed by glucose and identified as glucose-inhibited (glucose-INH) neurons; 11 were activated and identified as glucose-excited (glucose-EXC) neurons. Of 26 glucose-inhibited neurons examined for response to ghrelin, 23 were depressed, 1 was activated, and 2 failed to respond to ghrelin. Nine of 11 glucose-excited neurons were suppressed by ghrelin application, and the responses are abolished by the pretreatment with the GHS-R antagonist, [D-Lys-3]-GHRP-6. In addition, of 47 DVC neurons examined for responses to gastric distension (GD), 25 were excited (GD-EXC), 18 were inhibited (GD-INH). 18 out of the 25 GD-EXC neurons were excited, whereas 15 out of 18 GD-INH neurons were suppressed by ghrelin. In conclusion, the activity of the glucosensing neurons in the DVC can be modulated by ghrelin, the primary effect of ghrelin on the glucose-INH and glucose-EXC neurons was inhibitory. Two distinct population of GD-sensitive neurons exist in the rat DVC: GD-EXC neurons are activated by ghrelin; the GD-INH neurons are suppressed by ghrelin. There is a diversity of effects of ghrelin on neuronal activity within the DVC, it is as yet unclear how this diversity in ghrelin's effects on cellular excitability contributes to ghrelin biological actions to influence food intake and gastric motility.

Keywords: Ghrelin; Dorsal vagal complex (DVC); Glucose-sensing neuron; Gastric distension; Electrophysiology; Micro-pressure injection

1. Introduction

Ghrelin, which has recently been identified as the endogenous ligand of the growth hormone secretagogue receptor (GHS-R) [1], is a 28-amino acid peptide produced predominantly in the stomach. In addition to stimulation of growth hormone secretion, ghrelin also stimulates gastric motility in rodents and humans [2,3], and stimulate food intake and increase body weight and adiposity [4–6]. Although it is not yet clear whether endogenously produced ghrelin affects food intake by acting directly in the central nervous system or by acting in the periphery to alter vagal afferent firing [7], it appears likely that endogenous ghrelin is a "hunger hormone" that signals animals to take a meal. The primary action site of ghrelin seems to be the arcuate nucleus (ARC) of the hypothalamus. The ARC is located at the base of the hypothalamus on both sides of the third ventricle and, owing to the weak blood-brain barrier in this region of the brain, it is exposed to the peripheral signals, such as leptin, insulin and ghrelin [8,9]. Within the ARC, GHS receptors are expressed in 94% of neuropeptide Y (NPY)-containing neurons [10], and several studies have shown that both central and systemic administration of ghrelin leads to activation of NPY/agouti-related peptide (AgRP) neurons within the ARC [4,11]. Although there is strong evidence supporting a hypothalamic mode of action, there is also evidence that brainstem areas, in

Abbreviations: ARC, arcuate nucleus of the hypothalamus; AP, area postrema; DVC, dorsal vagal complex; GHS-R, growth hormone secretagogue receptor; NTS, nucleus of the solitary tract; NPY, Neuropeptide Y; AgRP, agouti-related peptide; PVN, paraventricular nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus.

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particular the nucleus of the solitary tract (NTS), play a crucial role in the control of food intake by conveying viscerosensory information about the gastrointestinal food content to higher regulatory brain centers [12,13]. With the new evidence provided by Faulconbridge et al. [14], ghrelin (150 pmol) delivered to the third and fourth ventricles significantly and comparably increased cumulative food intake, and ghrelin (10 pmol) microinjected unilaterally into the dorsal vagal complex (DVC) significantly increased food intake measured 1.5 and 3 h after the treatment. At the same time, it is worth noting that in central nervous system GHS receptor expression is not limited to the ARC [15,16]. A resent study by J.M. Zigman using in situ hybridization demonstrated the presence of GHS receptor in all three components of the DVC [17]. These findings provide the convincing evidence to support the view that there are both forebrain and hindbrain contributions to ghrelin-induced hyperphagia [18,19].

It is well established that the regulation of glucose is so important that the brain has glucose-sensing neurons located in key areas such as the hypothalamus, NTS, and amygdala which monitor glucose status in the body and initiate glucoprivic feeding, appropriate sympathoadrenal and neurohumoral responses to maintain homeostasis [20,21]. Other works have shown that hindbrain catecholaminergic neurons are glucose-responsive [22], glucose-excited neurons (glucose-EXC) increase and glucose-inhibited neurons (glucose-IHI) decrease their firing rate when extracellular glucose concentration rises [20,21]. Furthermore, hindbrain catecholaminergic neurons contribute to basal NPY and AgRP gene expression and mediate the responsiveness of NPY and AgRP neurons to glucose deficit [21,22]. We have previously shown that the activity of the glucose-INH neurons in the lateral hypothalamic area (LHA) and PVN, and the glucose-EXC neurons in the ventromedial nucleus of the hypothalamus (VMH) are modulated by the administration of ghrelin [23].

The aim of the present study was to identify the DVC glucosensing neurons, and to test the changes in firing rate of these cells responding to local administration of ghrelin. Extensive studies have demonstrated that the NTS in the caudal brainstem of the rat is the primary neuroanatomical site receiving visceral afferent information from postoral regions of the alimentary tract critical for the negative feedback control of food intake [24]. A separated group of experiments was done to identify the NTS neurons that responded to gastric distension (GD). By use of this approach, we hoped to see whether ghrelin could modulate the response of NTS neurons to gastric distention as well as gastric vago-vagal reflex.

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. A thermostatically controlled heating pad was used to maintain rectal temperature between 36–38 °C during the experiments.

2.2. 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. Removing a portion of the occipital bone and cerebellum exposed the dorsal surface of brainstem. Dura mater and arachnoid of the exposed medulla were carefully removed and 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 [23]. 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), 1.5×10^{-8} M solution of ghrelin, and 2.8×10^{-8} M 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 was purchased from American Peptide Company (Sunnyvale, CA 94086, USA), and the ghrelin receptor antagonist [D-Lys-3]-GHRP-6 was purchased from Sigma (D-91625 Schnelldorf, Germany). Drugs were ejected on the surface of firing cells with short pulse gas pressure (1500 ms, 5.0–15.0 psi) [25]. The intrabarrel drug concentrations were chosen on the basis of their efficacy to reliably alter cell firing. Less than 1 nl of

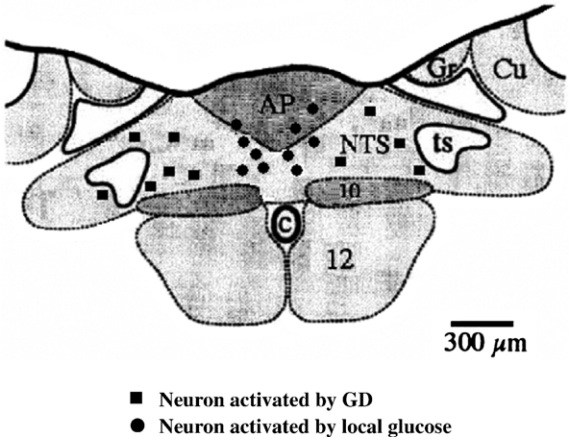

Fig. 1. Reconstruction of the dorsal medullary recording sites showing the location of some recorded neurons (−13.8 mm from bregma). AP, area postrema; C, central canal; Cu, nucleus cuneatus; Gr, nucleus gracilis; ts, tractus solitarii; 10, motor nucleus of the vagus nerve; 12, nucleus of the hypoglossal nerve.