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Electrophysiological effect of ghrelin and somatostatin on rat hypothalamic arcuate neurons in vitro

Kyohei Mori, Juhyon Kim, Kazuo Sasaki*

Division of Bio-Information Engineering, Faculty of Engineering, University of Toyama, 3190 Gofuku, Toyama City, Toyama 930-8555, Japan

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ABSTRACT

Growth hormone (GH) secretion from the pituitary gland is partly regulated by GH releasing hormone (GHRH)-containing neurons located in the hypothalamic arcuate nucleus (ARC). GHRH-containing neurons express the GH secretagogue (GHS) receptor (GHS-R) and the somatostatin (SRIF) receptor. Recently, an endogenous ligand for the GHS-R named ghrelin was found. Therefore, it seems that both ghrelin and SRIF are involved in the hypothalamic regulation of GH release via GHRH-containing neurons in the ARC. In extracellular single unit recordings from in vitro hypothalamic slice preparations from rats, application of 100 nM ghrelin substantially excited ARC neurons (82.5%), whereas 1 μM SRIF substantially inhibited them (81.8%). The ghrelin-induced excitatory and SRIF-induced inhibitory effects on ARC neurons were dose-dependent and persisted during synaptic blockade using low-Ca^{2+}/high-Mg^{2+} solution. In addition, the effects were antagonized by [D-Lys^3]-GHRP-6, a GHS-R antagonist, and CYN154806, a SRIF receptor subtype sst2 antagonist, respectively. When ghrelin and SRIF were sequentially applied to ARC neurons, 95.2% were excited by ghrelin and inhibited by SRIF. Similarly, 85.0% of ARC neuroendocrine cells that project to the median eminence were excited by ghrelin and inhibited by SRIF. These results indicate that ARC neuroendocrine cells projecting to the median eminence are dose-dependently, postsynaptically and oppositely regulated by ghrelin through GHS-R and SRIF via the SRIF sst2 receptor subtype. Our results also suggest that most of these ARC neuroendocrine cells are presumably GHRH-containing neurons and are involved in the cellular processes through which ghrelin and SRIF participate in the hypothalamic regulation of GH release.

1. Introduction

Growth hormone secretagogues (GHSs) are a family of small synthetic peptidyl and nonpeptidyl molecules that stimulate growth hormone (GH) secretion from the anterior pituitary gland in animals and humans [3,31,32]. Actions of GHSs are mediated through a specific receptor called the GHS receptor (GHS-R). This receptor is G-protein coupled and is distinct from the GH releasing hormone (GHRH) receptor [17,40]. Recently an endogenous ligand for the GHS-R called ghrelin was identified. Ghrelin displays strong GH releasing activity and is produced in brain tissues such as the hypothalamus as well as peripheral tissues such as the stomach [20]. GHS-Rs are mainly distributed in the pituitary gland and the hypothalamus, including the arcuate nucleus (ARC) [11,17,30,46]. In the ARC, a subpopulation of GHRH mRNA-containing neurons expresses the GHS-R gene [36,37]. These findings indicate that ghrelin and GHSs act at the pituitary and hypothalamic levels to release GH. However, several lines of evidence suggest that the most important action of ghrelin and GHSs for GH secretion takes place at the hypothalamic level through the mediation of GHRH-containing neurons [15,26,40].

GH secretion is also regulated by somatostatin (somatotropin release-inhibiting factor, SRIF). SRIF-containing neurons in the periventricular nuclei project to the median eminence (ME) and inhibit GH release via SRIF secretion in the pituitary portal system [4]. SRIF-containing neurons also project to the ARC [7,19], and GHRH-containing neurons in the ARC express the SRIF receptor subtypes sst1 and sst2 mRNA [33,38]. Thus, it seems likely that ghrelin and SRIF regulate electrical activity of GHRH-containing neurons in the ARC that express the GHS-R and/or the SRIF receptor, thereby modulating GH release. However, electrophysiological effects of ghrelin and SRIF on ARC neurons have not been described thoroughly. The aim of the present study was to examine whether both ghrelin and SRIF directly alter electrical activity of ARC neurons and to determine whether ARC neurons responding to these peptides are neuroendocrine cells that project to the ME.

* Corresponding author. Tel.: +81 76 445 6719; fax: +81 76 445 6723.
E-mail address: sasaki@eng.u-toyama.ac.jp (K. Sasaki).

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2. Materials and methods

2.1. Animals

Male Wistar rats (Sankyo Lab., Shizuoka, Japan) 12–16 days of age were used. Rats were housed with their mothers in a light-controlled room, light on 06:00–18:00, at a temperature of 23 ± 1 °C for several days before use in the experiments. Food and water were available ad libitum. The animals and experimental procedures used were approved by the Institutional Animal Care and Use Committee of the University of Toyama.

2.2. Slice preparation

After sevoflurane anesthesia was applied, the rats were decapitated, and their brains were rapidly removed from the skull. The brains were submerged in ice cold, oxygenated (95% O_2 and 5% CO_2) standard artificial cerebrospinal fluid (ACSF), which comprises (in mM) the following: NaCl 126, KCl 3, $CaCl_2$ 2.4, $MgSO_4$ 1.3, KH_2PO_4 1.25, $NaHCO_3$ 26 and glucose 10 with a pH of 7.4. Frontal hypothalamic slices 400 μm thick were cut using a microslicer (ZERO 1, Dosaka EM, Kyoto, Japan). Slices including the ARC were selected and cut with a scalpel along the third ventricle so that two ARC slices could be obtained from each frontal slice. However, when the ME was stimulated as described below, the frontal slice was not cut into two ARC slices. The slices containing the ARC were then preincubated in a chamber with oxygenated ACSF for about 1 h at room temperature.

2.3. Extracellular recording

After preincubation, the slices were transferred into a recording chamber and perfused with oxygenated standard ACSF at 1 ml/min and at 34 °C. Standard ACSF containing 5 mM KCl and 124 mM NaCl instead of 3 mM KCl and 126 mM NaCl, respectively, was also used to depolarize neurons slightly and to increase spontaneous firing rate. Extracellular electrical activity was recorded from the ventromedial part of the ARC via a glass microelectrode, resistance 5–15 MΩ, filled with ACSF, and then fed into a main amplifier via a preamplifier. The ventromedial part was selected because c-Fos, an immediate-early gene protein product, was mainly induced in this area after intracerebroventricular or systemic administration of ghrelin [13,29,39]. The output of the main amplifier was monitored on an oscilloscope and recorded on a magnetic tape. The output was also fed into a pulse generator to convert into a pulse and then into a computer through an A/D converter. The computer calculated the firing rate of a neuron in spikes/s and then recorded and displayed it in a histogram on a computer screen as a function of time. The basal firing rate in each neuron was then calculated as an average for 3 min immediately before the application of ghrelin or SRIF and this value was subtracted from subsequent changes in firing rate after the application of ghrelin or SRIF. Response to ghrelin and SRIF was evaluated as the change of mean firing rate averaged during the first 3-min period. If the change in mean firing rate after the application of ghrelin and SRIF was greater than ± 20% compared with the basal firing rate, the drug was considered to be effective. To examine whether the recorded ARC neuron is a neuroendocrine cell that projects to the ME, a bipolar stimulating electrode (OH99-0006, Unique Medical, Osaka, Japan) was placed on the ME. Stimulation with single pulse and/or double pulse with an interval of 30 ms was applied through the electrode. Each stimulation pulse was a 0.5 mA monophasic current pulse with a duration of 0.3 ms. Neuroendocrine cells were identified by an occurrence of antidromic action potential with a constant latency following electrical stimulation and/or by a collision between spontaneous and antidromic action potentials [16,42]. In the present study, recordings were made from only one neuron in each slice.

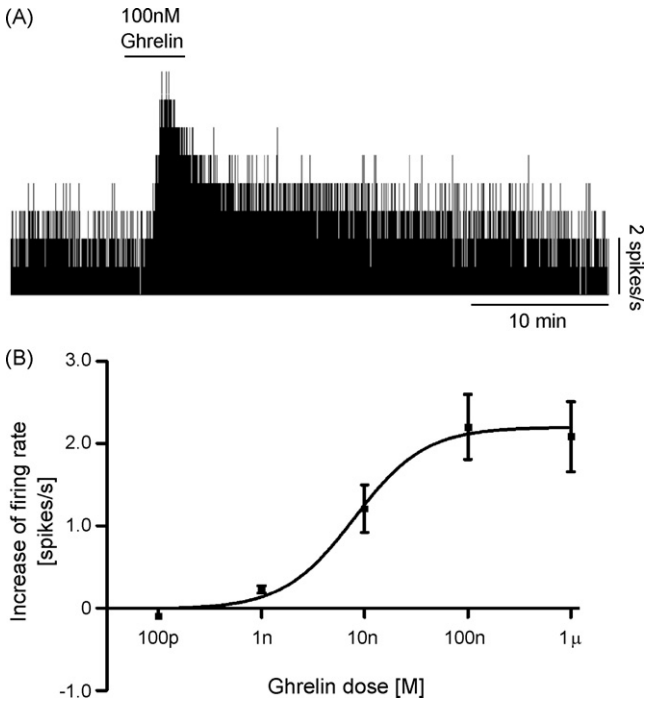

Fig. 1. Dose-dependent excitatory effect of ghrelin on ARC neurons. (A) Excitatory neuronal activity during treatment with 100 nM ghrelin (solid bar). (B) Dose–response curve of ARC neuronal activity versus increasing concentrations of ghrelin; means ± SEMs. The number of neurons tested for each concentration was 5 for 100 pM, 6 for 1 nM, 6 for 10 nM, 11 for 100 nM and 7 for 1 μM.

2.4. Drugs

Ghrelin (Peptide Institute, Osaka, Japan), SRIF (Sigma, Tokyo, Japan), [D-Lys^3]-GHRP-6 (Sigma) and CYN154806 (Sigma) were dissolved in saline and stored at −40 °C in small aliquots. All stock solutions were then diluted with ACSF to desired concentrations just before use in the experiments and were added to the perfusate.

2.5. Statistics

All data are expressed as means ± SEMs. For multiple comparisons, one-way analysis of variance (ANOVA) with repeated measures followed by Fisher's least significant difference (LSD) test was used. Student's *t*-test and Fisher's exact test were also used. Level of statistical significance was taken as $p < 0.05$.

3. Results

To examine the effect of ghrelin and SRIF on ARC neurons, extracellular recording was done in acute slice preparations from rats. In total, 204 neurons were recorded, and the mean spontaneous firing rate of these neurons was 1.18 ± 0.07 spikes/s ($n = 204$).

3.1. Excitatory effects of ghrelin on ARC neurons and dose–response curve

Of 57 ARC neurons that were challenged by 100 nM ghrelin, 47 (82.5%) were excited (Fig. 1A), and the remaining 10 (17.5%) were unchanged. None of the neurons were suppressed by ghrelin. The mean latency, duration and firing rate for ghrelin-induced excitation obtained from 47 neurons were 101.0 ± 7.8 s ($n = 47$),