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Research article

Analysis of peripheral ghrelin signaling via the vagus nerve in ghrelin receptor–restored GHSR-null mice

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ABSTRACT

The vagus nerve connects peripheral organs to the central nervous system (CNS), and gastrointestinal hormones transmit their signals to the CNS via the vagal afferent nerve. Ghrelin, a gastric-derived orexigenic peptide, stimulates food intake by transmitting starvation signals via the vagus nerve. To understand peripheral ghrelin signaling via the vagus nerve, we investigated the ghrelin receptor (GHSR)-null mouse. For this purpose, we tried to produce mice in which GHSR was selectively expressed in the hindbrain and vagus nerve. GHSR was expressed in some nodose ganglion neurons in these mice, but GHSR-expressing neurons were less abundant than in wild-type mice. Intraperitoneal administration of ghrelin did not induce food intake or growth hormone release, but did increase blood glucose levels. Our findings suggest that the abundance of GHSR-expressing neurons in the nodose ganglion is critical for peripheral administration of ghrelin-induced food intake and growth hormone release via the vagus nerve.

1. Introduction

The vagus nerve, one of the cranial nerves, conveys signals from the gastrointestinal tract to the central nervous system (CNS). The appetite-stimulating hormone ghrelin, a 28–amino acid peptide, is produced in gastric endocrine cells [1]. Two forms of ghrelin exist, *n*-octanoylated ghrelin and desacyl-ghrelin. The ghrelin receptor (also known as the growth hormone secretagogue receptor, GHSR) is produced in nodose ganglion neurons and transferred to the stomach via axons [2]. The ghrelin signal is transmitted to the nucleus tractus solitarii (NTS) in the medulla oblongata via the vagus afferent nerve, and then relayed to hypothalamic neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP) [3].

A previous report demonstrated that GHSR restoration in the hindbrain is not sufficient to induce ghrelin-stimulated food intake in GHSR-null mice [4]. However, this study did not investigate the ghrelin signaling via the vagal afferent nerve. In the current study, we studied peripheral ghrelin signaling via the vagus nerve in GHSR-null mice and in GHSR/Phox2b mice, which express GHSR only in the nodose ganglion neurons. Restoration of GHSR expression in nodose ganglion neurons rescued low fasting blood glucose levels in GHSR-null mice. Intraperitoneal administration of ghrelin elevated blood glucose levels, but did not induce food intake or growth hormone release in GHSR/Phox2b mice. GHSR-positive neurons in the nodose ganglion were less abundant in GHSR/Phox2b mice than in wild-type mice. Our results suggest that GHSR neurons expressed in the nodose ganglion play a critical role in gastric-derived ghrelin signaling.

2. Materials and methods

2.1. Animals

Mice were maintained in cages under controlled temperature (21–23 °C) and light conditions (light on: 08:00–20:00). Animals were maintained on a chow diet (CLEA Rodent Diet CE-2; CLEA Japan). GHSR-null mice [5], which harbor a loxP-flanked transcriptional blocking cassette (TBC) in both endogenous GHSR alleles, were provided by Syu Takeda at Tokyo Medical and Dental University [6].

Abbreviations: CNS, central nervous system; GLP-1, glucagon-like peptide-1; GHSR, growth hormone secretagogue receptor; NTS, nucleus tractus solitarii; NPY, neuropeptide Y; AgRP, agouti-related peptide; TBC, transcriptional blocking cassette

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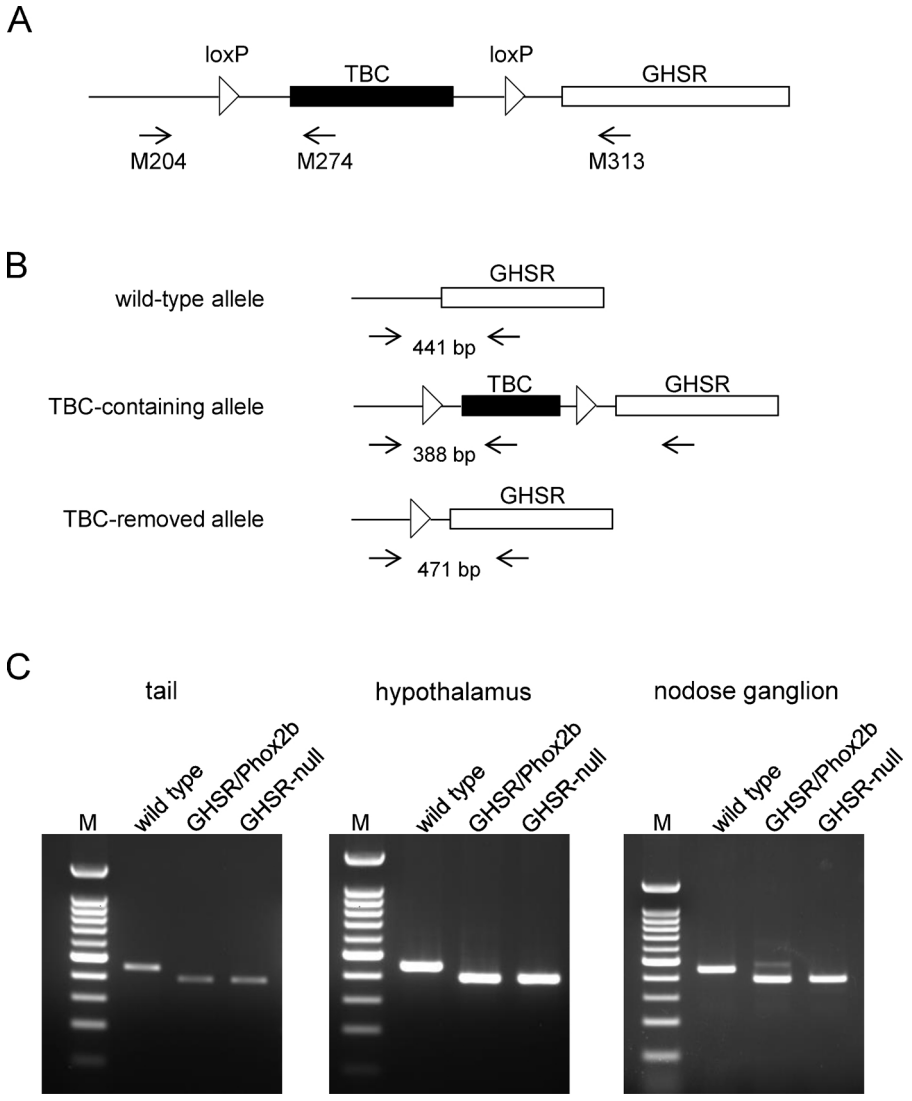

Fig. 1. Confirmation of tissue-specific removal of the loxP-flanked TBC by genomic PCR. (A, B) Schematic illustration of GHSR alleles in the GHSR-null mouse. Arrows show primer binding sites. (B) Illustration of primer binding sites and amplified PCR product sizes in wild-type, GHSR-null, GHSR/Phox2b (TBC not removed), and GHSR/Phox2b (TBC removed). (C) PCR products from tail, hypothalamus, and nodose ganglion.

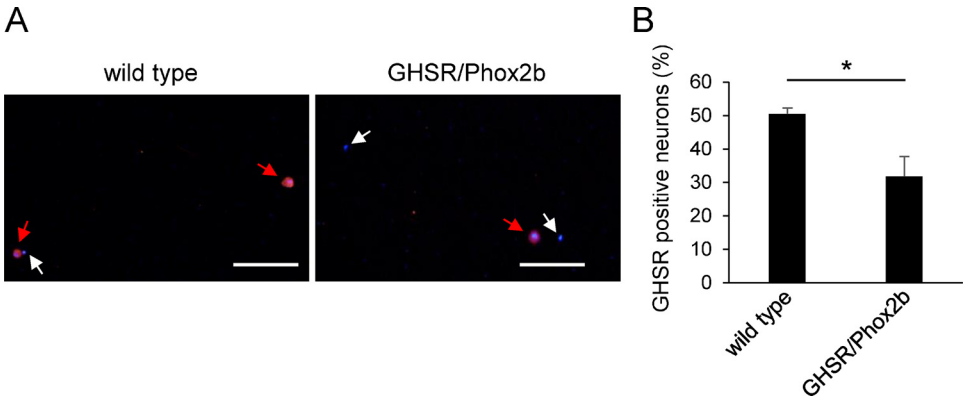

Fig. 2. Ghrelin–rhodamine binding of GHSR-expressing neurons. (A) Representative fluorescence images of ghrelin–rhodamine binding of GHSR-restored neurons. Red arrows indicate GHSR-positive neurons, and white arrows indicate GHSR-negative neurons. Nuclei (blue) were stained with DAPI. Scale bars, 100 µm. (B) Frequency of GHSR-restored neurons in wild-type and GHSR/Phox2b mice. Values are means ± SEM. *$P < 0.05$ vs wild-type mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Phox2b-Cre mice, which express Cre recombinase in the dorsal vagal complex and vagal sensory neurons [7], were purchased from The Jackson Laboratory (USA). Breeding of GHSR-null mice with Phox2b-Cre mice led to removal of the loxP-flanked TBC in offspring, resulting in tissue-specific restoration of GHSR expression. All animal experiments were approved by the Animal Care and Use Committee of the University of Miyazaki.

2.2. Genotyping

Tail tips were sampled from 4-week-old transgenic mice and used for genotyping. Nodose ganglion and hypothalamus were taken from