Biochemical and Biophysical Research Communications 411 (2011) 445–448

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The contribution of serotonin 5-HT2C and melanocortin-4 receptors to the satiety signaling of glucagon-like peptide 1 and liragultide, a glucagon-like peptide 1 receptor agonist, in mice

Katsunori Nonogaki [a,*], Marina Suzuki [a], Marin Sanuki [a], Mamoru Wakameda [b], Tomohiro Tamari [b]

[a] Department of Lifestyle Medicine, Biomedical Engineering Center, Tohoku University, Japan
[b] Charles River Laboratories Japan Inc., Japan

ARTICLE INFO

Article history:
Received 27 June 2011
Available online 2 July 2011

Keywords:
GLP-1
Liraglutide
GLP-1 receptor
5-HT2C receptor
Melanocortin-4 receptor
Food intake
Body weight

ABSTRACT

Glucagon-like peptide 1 (GLP-1), an insulinotropic gastrointestinal peptide produced mainly from intestinal endocrine L-cells, and liraglutide, a GLP-1 receptor (GLP-1R) agonist, induce satiety. The serotonin 5-HT2C receptor (5-HT2CR) and melanoroctin-4 receptor (MC4R) are involved in the regulation of food intake. Here we show that systemic administration of GLP-1 (50 and 200 μg/kg)-induced anorexia was blunted in mice with a 5HT2CR null mutation, and was attenuated in mice with a heterozygous MC4R mutation. On the other hand, systemic administration of liraglutide (50 and 100 μg/kg) suppressed food intake in mice lacking 5-HT2CR, mice with a heterozygous mutation of MC4R and wild-type mice matched for age. Moreover, once-daily consecutive intraperitoneal administration of liraglutide (100 μg/kg) over 3 days significantly suppressed daily food intake and body weight in mice with a heterozygous mutation of MC4R as well as wild-type mice. These findings suggest that GLP-1 and liraglutide induce anorexia via different central pathways.

1. Introduction

Glucagon-like peptide 1 (GLP-1) is an incretin hormone released from intestinal L-cells in response to nutrient ingestion [1]. GLP-1 potentiates glucose-dependent insulin secretion by activating the GLP-1 receptor (GLP-1R) expressed on pancreatic islet β-cells [1–3]. GLP-1R signaling increases β-cell sensitivity to glucose and decreases blood glucose in type 2 diabetes [2–4]. GLP-1R is also expressed in the central nervous system, and the extrapancreatic functions of GLP-1 include its effects on the hypothalamus promoting satiety [1,2,5].

Once released from L-cells into the bloodstream, GLP-1 is rapidly degraded from its active form GLP-1 (7–36) to the inactive, *n*-terminally truncated form GLP-1 (9–36) by dipeptidyl peptidase-4 (DPP-4) [1–3]. The discovery of structurally distinct GLP-1R agonists, which are resistant to degradation by DPP-4 and have an increased circulation half-life, have led to a mimicking of GLP-1 activity *in vivo* [1–3].

Liraglutide activates the GLP-1R, leading to insulin release in the presence of elevated glucose concentrations, and it decreases glucagon secretion in a glucose-dependent manner [2,3]. The mechanism of blood glucose lowering also involves a delay in gastric emptying [2]. Liraglutide decreases food intake and body weight in normal and obese rats, minipigs and humans [6–9]. However, the mechanism by which GLP-1 and GLP-1R induce satiety remains unclear.

Brain serotonin (5-hydroxytryptamine; 5-HT) systems contribute to the regulation of food intake. The 5-HT2C receptor (5HT2CR) has a major role in the leptin-independent regulation of energy intake [10]. Systemic administration of GLP-1 (33-132 μg/kg) suppresses food intake for 30 min in wild-type mice, whereas the anorexic effects are attenuated in 5-HT2C receptor mutant mice [11]. These findings suggest that central 5-HT2C receptors substantially contribute to the anorexic effect of GLP-1. 5-HT2C receptors are expressed on POMC neurons in the hypothalamus, and the melnocortin-4 receptor (MC4R) is reportedly active downstream of 5-HT2C receptor satiety signaling [12–14].

To determine whether the functional 5-HT2C receptor and MC4R activities are required for the satiety induced by GLP-1 and GLP-1R stimulation, we examined the effects of systemic GLP-1 or liraglutide administration on food intake in mice with a 5-HT2C receptor null mutation, mice with a heterozygous mutation of MC4R and wild-type mice matched for age.

* Corresponding author. Address: Department of Lifestyle Medicine, Biomedical Engineering Center, Tohoku University, 1-1 Seiryou-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan. Fax: +81 22 717 7061.
E-mail addresses: katsu@trc.med.tohoku.ac.jp, knonogaki-tky@umin.ac.jp (K. Nonogaki).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.06.175

To further determine whether functional MC4R activity is required for the liraglutide effect on food intake and body weight, we examined once-daily consecutive intraperitoneal administration of liraglutide over 3 days on food intake and body weight in mice with a heterozygous mutation of MC4R and wild-type mice matched for age.

2. Materials and methods

2.1. Mice

Hemizygous mutant males bearing a null mutation of the X-linked htr2c gene (congenic on a C57BL/6J background) and age-matched wild-type mice were used. The line is maintained through the mating of females heterozygous for the htr2c gene with C57BL/6J males obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Genotypes were confirmed by Southern blot analysis of BamHI digested genomic DNA from the tail of litter-mates, as described previously [15]. Blots were hybridized with a 3k-flanking probe. The wild-type and htr2c mutant alleles correspond with the 6.0-kb and 2.5-kb fragments, respectively.

Heterozygous mutant males bearing a null mutation of the MC4R gene (congenic on a C57BL/6J background) and age-matched wild-type mice were also used. The line is maintained through the mating of females heterozygous for the MC4R gene with heterozygous males obtained from the Jackson Laboratory (Stock# 006414). Genotypes were confirmed by Southern blot analysis of Dra I-digested genomic DNA from the tails of litter-mates. Blots were hybridized with a 567 bp 5′-flanking probe. The wild-type and MC4R mutant alleles correspond with the 2.5-kb and 5.0-kb fragments, respectively. The animals were housed in individual cages with free access to water and chow pellets on a 12-h light–dark cycle (lights off 20:00 h) in a temperature-controlled (20–22 °C) environment.

In the first experiment, 6-month-old male 5-HT2CR mutant mice, mice with a heterozygous mutation of MC4R and wild-type mice were intraperitoneally injected with saline, GLP-1 (50 and 200 μg/kg) or liraglutide (50 and 100 μg/kg) 30 min before the onset of the dark cycle. Chow pellets were provided 30 min later. The intake of chow pellets was measured for the next 1 h and then 2 h after the onset of the dark cycle.

In the second experiment, 6-month-old mice with a heterozygous mutation of MC4R and wild-type mice were once-daily intraperitoneally injected consecutively with saline or liraglutide (100 μg/kg) over 3 days. Body weight and daily food intake were measured on the first, second, and third days after the injection. The experiment was carried out at the time of 15:00–16:00.

The doses of GLP-1 (50 and 200 μg/kg) were selected based on the evidence that GLP-1-induced hypophagia was attenuated by the genetic blockade of 5-HT2CR [11]. The doses of liraglutide (50 and 100 μg/kg) were selected based on the evidence that liraglutide induces hypophagia [6,7]. Human GLP-1 (7–36) amide was purchased from Bachem Inc. (Torrance, CA, USA). Liraglutide was a kind gift from Novo Nordisk, Japan. The drugs were dissolved in 0.2 ml 0.9% saline.

The animal studies were conducted in accord with the institutional guidelines for animal experiments at the Tohoku University Graduate School of Medicine.

2.2. Statistical methods

Data are presented as the mean values ± SEM (n = 8–12). Comparisons among more than two groups were performed with ANOVA using Bonferroni's test. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of GLP-1 or liraglutide on food intake in wild-type mice, mice with a null 5-HT2CR mutation or a heterozygous mutation of MC4R

Systemic administration of GLP-1 (50 and 200 μg/kg) significantly suppressed food intake compared with saline in wild-type

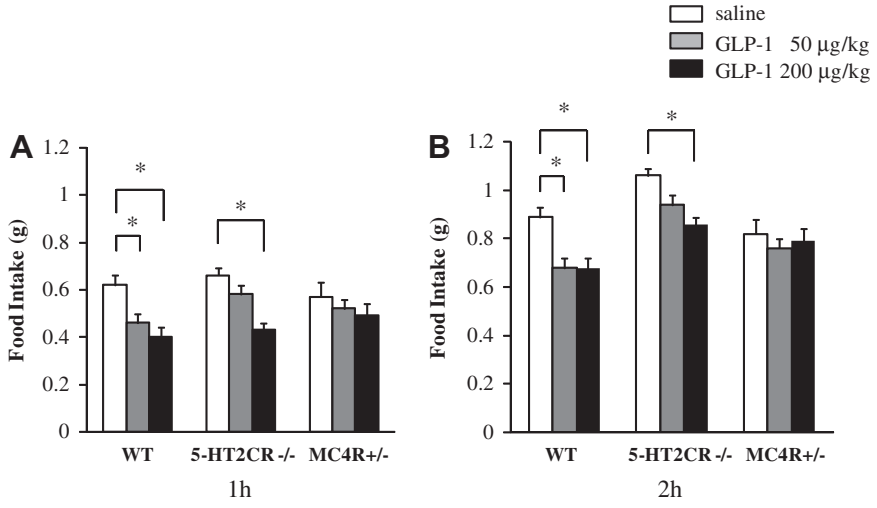

Fig. 1. Effects of systemic administration of GLP-1 (50 and 200 μg/kg) or saline on food intake in wild-type mice, 5-HT2CR mutant mice, or mice with a heterozygous mutation of MC4R. The intake of chow pellets was measured for the next hour and then 2 h after the onset of the dark cycle, as described in the Section 2. The basal body weights in the wild-type mice, 5-HT2CR mutants and MC4R heterozygous mutants treated with saline were 34.8 ± 0.1 g, 34.6 ± 0.2 g and 49.2 ± 0.2 g, respectively. The basal body weights in the wild-type mice, 5-HT2CR mutants and MC4R heterozygous mutants treated with GLP-1 (50 μg/kg) were 34.6 ± 0.2 g, 34.4 ± 0.2 g and 46.6 ± 0.2 g, respectively. Body weights in the wild-type mice, 5-HT2CR mutants and MC4R heterozygous mutants treated with GLP-1 (200 μg/kg) were 33.9 ± 0.2 g, 34.6 ± 0.2 g and 46.7 ± 0.2 g, respectively. Body weights in the wild-type mice, 5-HT2CR mutants and MC4R heterozygous mutants treated with saline were 33.9 ± 0.1 g, 35.1 ± 0.2 g and 45.6 ± 0.2 g, respectively. WT, wild-type mice; 5-HT2CR−/−, mice with null mutation of 5-HT2CR; MC4R+/−, mice with a heterozygous mutation of MC4R; C, saline controls. Data are presented as the mean values ± SEM (n = 6–12 for each group of animals). *P < 0.05.