Biochemical and Biophysical Research Communications 420 (2012) 36–41

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Possible involvement of melanocortin-4-receptor and AMP-activated protein kinase in the interaction of glucagon-like peptide-1 and leptin on feeding in rats

Paul-Emile Poleni [a,1], Sayaka Akieda-Asai [a,1], Shuichi Koda [b], Maya Sakurai [a], Cho-Rong Bae [c], Kazuyo Senba [a], Youn-Soo Cha [c], Mayumi Furuya [b], Yukari Date [a,*]

[a] Frontier Science Research Center, University of Miyazaki, Miyazaki 889-1692, Japan
[b] Faculty of Pharmacology I, Asubio Pharma Co., Ltd., Kobe 650-0047, Japan
[c] Department of Food Science and Human Nutrition, Obesity Research Center, Chonbuk National University, Jeonju 561-756, Republic of Korea

ARTICLE INFO

Article history:
Received 18 January 2012
Available online 27 February 2012

Keywords:
GLP-1
Leptin
Feeding
MC4-R
AMPK

ABSTRACT

Glucagon-like peptide-1 (GLP-1) and leptin are anorectic hormones produced in the small intestine and white adipose tissue, respectively. Investigating how these hormones act together as an integrated anorectic signal is important to elucidate a mechanism to maintain energy balance. In the present study, coadministration of subthreshold GLP-1 and leptin dramatically reduced feeding in rats. Although coadministration of GLP-1 with leptin did not enhance leptin signal transduction in the hypothalamus, it significantly decreased phosphorylation of AMP-activated protein kinase (AMPK). In addition, coadministration of GLP-1 with leptin significantly increased proopiomelanocortin (POMC) mRNA levels. Considering that α-melanocortin stimulating hormone (α-MSH) is derived from POMC and functions through the melanocortin-4-receptor (MC4-R) as a key molecule involved in feeding reduction, the interaction of GLP-1 and leptin on feeding reduction may be mediated through the α-MSH/MC4-R system. As expected, the interaction of GLP-1 and leptin was abolished by intracerebroventricular preadministration of the MC4-R antagonists agouti-related peptide and SHU9119. Taken together, GLP-1 and leptin cooperatively reduce feeding at least in part via inhibition of AMPK following binding of α-MSH to MC4-R.

1. Introduction

The body weights of adult animals or humans are essentially maintained within a fixed range, even if the daily amount of food consumption is changed [1,2]. To sustain the body weight in this way, multiple signals such as nutrients or hormones must interact to efficiently regulate energy balance [3].

Leptin is produced by white adipose tissue and maintains energy balance by reducing feeding. It binds to its receptor, ob-Rb, and stimulates the Janus-activated kinase (JAK)/signal transducer and activator of transcription-3 (STAT3) signaling pathway. Many of the neuropeptides that regulate feeding are closely associated with the leptin signaling pathway in the hypothalamus [4,5]. Furthermore, leptin administration enhances the anorectic response to satiety signaling molecules such as cholecystokinin (CCK), bombesin, or amylin [6–8]. Williams et al. also demonstrated that a subthreshold dose of leptin enhances the feeding reduction and weight loss induced by glucagon-like peptide-1 (GLP-1) [9]. GLP-1 is a gastrointestinal hormone produced by L cells in the small intestine and is thought to function as a satiety signal. This hormone is released in response to meal intake [10] and acts on the GLP-1 receptor (GLP-1R) that is present in vagal afferents, pancreatic β-cells, and neurons in the central nervous system (CNS; see Refs. [11,12] for review).

In this study, we investigated whether GLP-1 and leptin have interactive effects on feeding using overnight fasted rats that were administered subthreshold doses of GLP-1 and leptin. Furthermore, we examined leptin signal transduction and the phosphorylation of AMP-activated protein kinase (AMPK) in the hypothalamus after coadministration of GLP-1 and leptin. We also evaluated mRNA expression of hypothalamic substances relative to feeding such as neuropeptide Y (NPY), agouti-related protein (AGRP), cocaine- and amphetamine-regulated transcript (CART), and proopiomelanocortin (POMC). To clarify molecular mechanisms in the hypothalamus

Abbreviations: AGRP, agouti-related protein; AMPK, AMP-activated protein kinase; α-MSH, α-melanocyte-stimulating hormone; CART, cocaine-and amphetamine-regulated transcript; CCK, cholecystokinin; CNS, central nervous system; GLP-1, glucagon-like peptide-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLP-1R, GLP-1 receptor; ip, intraperitoneal or intraperitoneally; HRP, horseradish peroxidase; icv, intracerebroventricular or intracerebroventricularly; JAK, janus-activated kinase; MC4-R, melanocortin-4-receptor; NPY, neuropeptide Y; NTS, nucleus tractus solitarius; pAMPK, phosphorylated AMPK; POMC, proopiomelanocortin; pSTAT3, phosphorylated STAT3.

* Corresponding author. Address: Frontier Science Research Center, University of Miyazaki, Kiyotake, Miyazaki 889-1692, Japan.
E-mail address: dateyuka@med.miyazaki-u.ac.jp (Y. Date).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2012.02.109

involved in the interaction of GLP-1 and leptin, we investigated whether the interactive effect of these hormones on feeding is abolished in rats that were preadministered antagonists for hypothalamic substances.

2. Materials and methods

2.1. Experimental animals

Male Wistar rats (10 weeks old; Charles River Japan, Inc., Shiga, Japan), weighing 300–350 g, were used for all experiments. Rats were given standard laboratory chow and water *ad libitum* and housed individually in plastic cages at constant room temperature in a 12-h light/12-h dark cycle (0800–2000 h light). Anesthesia was performed with an ip injection of sodium pentobarbital (40 mg/kg; Abbot Laboratories, Chicago, IL, USA) before the following operations. For MC4-R blockade, we implanted intracerebroventricular (icv) cannulae into the lateral cerebral ventricles of rats after anesthesia. Proper placement of the cannula was verified by dye injection at the end of the experiment. Only animals exhibiting progressive weight gain after surgery were used in experiments. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care. This protocol was approved by the Ethics Review Committee for Animal Experimentation of the Faculty of Medicine, University of Miyazaki.

2.2. Feeding experiments

We evaluated the dose-dependency of GLP-1 and leptin on feeding. A solution of rat recombinant leptin (Sigma Aldrich, Saint Louis, MI, USA) dissolved in 0.9% saline (18.8–62.5 nmol/kg in a volume of 100 μL) was administered ip at 0930–1000 h to rats that had fasted overnight (n = 6–10). GLP-1 (7-36) amide (Peptide Institute, Inc., Osaka, Japan) was dissolved in 0.9% saline, and this solution (10–40 nmol/kg in a volume of 100 μL) was administered ip at 0930–1000 h to another set of rats fasted overnight (n = 6–10). After the injection, cumulative food intake was measured at 0.5, 1, and 2 h. Next, to evaluate the interaction of GLP-1 and leptin on feeding reduction, we used the dosage of 10 nmol/kg GLP-1 and 18.8 nmol/kg leptin, which individually did not decrease feeding of rats fasted over night. Rats fasted overnight were divided into four groups; a saline-injected group, a group injected with GLP-1 (10 nmol/kg), a group injected with leptin (18.8 nmol/kg), and a group coinjected with GLP-1 (10 nmol/kg) and leptin (18.8 nmol/kg). We measured the food intake of each group at 0.5, 1, and 2 h after injections.

To evaluate the contribution of MC4-R signaling to the interaction of the hormones, rats fasted overnight were administered icv vehicle alone (saline; n = 6), or the MC4-R antagonists, AGRP (16 μg/kg; n = 5; Peptide Institute, Osaka, Japan), or SHU9119 (1.2 μg/kg; n = 5; Sigma Aldrich) 1 h before peripheral coinjection of GLP-1 (10 nmol/kg) and leptin (18.8 nmol/kg). These doses of AGRP or SHU9119 are known to prevent feeding reduction caused by α-melanocyte stimulating hormone (α-MSH) [13,14]. GLP-1 + leptin or saline was administered ip to rats 1 h after preadministration of saline, AGRP, or SHU9119, and food intake was measured after 0.5, 1, and 2 h. The rats were fasted between the two injections.

2.3. Western blotting

The rats were decapitated and their hypothalami were rapidly removed 0.5 h after ip injection of saline, GLP-1, leptin, or GLP-1 + leptin into rats (n = 5–6 per group), and the amount of total protein was measured for each sample by the bicinchoninic acid method (Pierce Microplate BCA Protein Assay Kit, Thermo Scientific, Rockford, IL, USA). 30 μg of total proteins per sample were analyzed by SDS-PAGE (8% acrylamide) and electroblotted onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, Tokyo, Japan). Membranes were then sequentially blocked in 5% (w/v) blocking buffer (5% (w/v) non-fat dry milk diluted in TBS Tween20), probed overnight at 4 °C with gentle shaking with primary antibody against Stat-3, phospho-Stat-3, AMPKα1 and 2, or phospho-AMPKα1 and 2 (Cell Signaling Technology, Inc., Danvers, MA, USA), and incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies against the molecular weight standard (Biotinylated Protein Ladder, Cell Signaling Technology, Inc.) and an HRP labeled goat anti-rabbit IgG (H + L) antibody (Epitomics, Inc., Burlingame, CA, USA). Specific proteins were detected by chemiluminescence with the Phototope Detection system (Cell Signaling Technology, Inc.) in accordance with the manufacturer's instructions. Western blotting was quantified by densitometry relative to total STAT3 or AMPK by using NIH Image software (Image J).

2.4. Quantitative PCR

The hypothalami of rats fasted overnight were removed 1.5 h after ip injection of saline, GLP-1 (10 nmol/kg), leptin (18.8 nmol/kg), or GLP-1 (10 nmol/kg) and leptin (18.8 nmol/kg) (n = 10 in each group), and total RNAs were rapidly extracted with TRIzol® Reagent (Invitrogen Corp., Carlsbad, CA, USA). First-strand cDNA was synthesized from 1 μg total RNA using the commercially available Superscript® III First-Strand Synthesis System kit (Invitrogen Corp.), and the resulting samples were subjected to quantitative PCR. Quantitative PCR for NPY, AGRP, CART, and POMC was conducted on a LightCycler system (Roche Diagnostics GmbH, Mannheim, Germany) using the SYBR® Premix Ex Taq™ mix system (Takara Bio Inc., Shiga, Japan) with primer sets described elsewhere [15]. The relative abundance of all reaction products was normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

2.5. Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical significance was evaluated by a one-way or a two-way ANOVA. When ANOVA indicated a significant effect of the variable, differences between groups were analyzed using Dunnett's or Bonferroni's post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Interaction of GLP1 with leptin on food intake

We first tested various doses of leptin from 18.8 to 62.5 nmol/kg and of GLP-1 ranging from 10 to 40 nmol/kg in a feeding experiment using rats fasted overnight. An ip injection of 62.5 nmol/kg leptin significantly decreased food intake 2 h after injection, whereas neither 18.8 nmol/kg nor 35.7 nmol/kg leptin reduced feeding (Fig. 1A). An ip injection of 40 nmol/kg GLP-1 significantly decreased food intake by 0.5, 1, and 2 h after injection; however, neither 20 nmol/kg nor 10 nmol/kg GLP-1 reduced feeding (Fig. 1B). Therefore, we examined the interaction of GLP-1 and leptin on feeding using 18.8 nmol//kg leptin and 10 nmol/kg GLP-1. An ip coinjection of 10 nmol/kg GLP-1 and 18.8 nmol/kg leptin significantly reduced food intake of overnight fasted rats 0.5 and 1 h after injection compared to saline, 10 nmol/kg GLP-1, or 18.8 nmol/kg leptin injection (Fig. 1C).