Biochemical and Biophysical Research Communications 492 (2017) 161–165

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Canagliflozin potentiates GLP-1 secretion and lowers the peak of GIP secretion in rats fed a high-fat high-sucrose diet

Tohru Hira [a, b, *], Toshiki Koga [b], Kazuyo Sasaki [c], Hiroshi Hara [a, b]

[a] Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan
[b] Graduate School of Agriculture, Hokkaido University, Sapporo, Japan
[c] Mitsubishi Tanabe Pharma Corporation, Osaka, Japan

ARTICLE INFO

Article history:
Received 28 July 2017
Accepted 9 August 2017
Available online 10 August 2017

Keywords:
Diet–induced obesity
Canagliflozin
Glucagon-like peptide-1
Glucose-dependent insulinotropic polypeptide
Sodium-glucose cotransporter 1(SGLT1)
SGLT2 inhibitor

ABSTRACT

The glucose-induced secretion of incretins, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), is dependent on luminal glucose levels and transport of glucose via the sodium-glucose transporter 1 (SGLT1) in the small intestine. Because GLP-1 and GIP function in decreasing and increasing the body weight, respectively, we aimed to analyze the effect of transient inhibition of SGLT1 by canagliflozin on incretin secretion in an obese rat model. Male Sprague-Dawley rats were maintained on a high-fat high-sucrose diet for 6–7 weeks, and plasma GLP-1 and GIP levels were measured during an oral glucose tolerance test (OGTT). In addition, GLP-1 secretion was examined in a murine GLP-1 producing enteroendocrine cell line, GLUTag. Concomitant administration of 10 mg/kg canagliflozin with glucose loading suppressed glucose excursion, increased total GLP-1 levels, and reduced total GIP levels in systemic circulation, as revealed in the OGTT. Total and active GLP-1 levels were increased in portal blood, whereas total and active GIP levels tended to be decreased 15 min after the administration of canagliflozin with glucose. Canagliflozin (at 0.1–30 μM) did not directly affect release of GLP-1 *in vitro*. These results suggest that the oral administration of canagliflozin suppresses GIP secretion via the inhibition of SGLT1 in the upper part of the intestine and enhances GLP-1 secretion by increasing the glucose delivery to the lower part of the small intestine in an obese rodent model.

1. Introduction

Obesity is a risk factor for development of type 2 diabetes mellitus (T2DM) and cardiovascular diseases, and current therapies to promote weight loss include dietary, exercise, drug therapies and gastric bypass surgery. Recent studies have indicated the potential of incretin-based therapies in obese subjects. Incretin hormones include glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are synthesized and secreted by enteroendocrine L and K cells, respectively, in response to nutrient stimulation.

GLP-1 exerts pleiotropic effects that induce satiety and inhibit gastric emptying. Administration of GLP-1-mimetics improves glycemic control accompanied by weight loss in patients with T2DM [1–3], and endogenous GLP-1 contributes to weight loss in dietary and exercise therapies and gastric bypass surgery [2]. In contrast, GIP promotes obesity; GIP receptor knock-out has been shown to prevent the development of obesity, and reduction of GIP secretion has been shown to ameliorate weight gain in high-calorie-diet-fed mice [3,4]. Luminal or oral administration of isomaltulose, a dietary peptide, and prebiotic fibers has been reported to promote GLP-1 secretion in rats [5–8], while effects of oral drugs on incretin secretions are not well known.

Canagliflozin is a sodium–glucose cotransporter 2 (SGLT2) inhibitor, which ameliorates hyperglycemia by promoting the urinary excretion of glucose by inhibition of renal SGLT2 [9–12]; it has a modest SGLT1 inhibitory activity [11]. SGLT1 is predominantly expressed in intestinal epithelia, plays a major role in the transport of luminal glucose into epithelial cells, and mediates the glucose-induced incretin secretion in the intestine [13–15]. Clinical

Abbreviations: DPP-IV, dipeptidyl peptidase-IV; GLP-1, glucagon-like peptide-1; GIP, Glucose-dependent insulinotropic poypeptide; HFS, high-fat high-sucrose; OGTT, oral glucose tolerance test; PYY, peptide-YY; SGLT, sodium–glucose cotransporter; T2DM, type 2 diabetes mellitus.

* Corresponding author. Laboratory of Nutritional Biochemistry, Research Faculty of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kita-ku, Sapporo 060-8589, Japan.
E-mail address: hira@chem.agr.hokudai.ac.jp (T. Hira).

http://dx.doi.org/10.1016/j.bbrc.2017.08.031
0006-291X/

studies have reported the efficacy of canagliflozin in reducing the body weight in obese patients with T2DM [10]. Despite its inhibitory effects on SGLT1, elevated plasma GLP-1 levels have been demonstrated in genetically diabetic rodents [16–18] and in healthy subjects [19]. Although the molecular structure of canagliflozin has been known to contain a glucoside group, its direct effect on GLP-1-producing cells remains unclear.

In the present study, to determine the effect of canagliflozin on incretin secretion in obesity, we measured endogenous GLP-1 and GIP levels in the systemic and portal circulation in rats that were fed a high-fat high-sucrose (HFS) diet, which is a model of diet-induced obesity [8,20]. Additionally, we examined the direct effect of canagliflozin on GLP-1 secretion in a GLP-1 producing murine cell line (GLUTag).

2. Materials and methods

2.1. Animals and diets

Five-weeks-old Male Sprague–Dawley rats were purchased from Japan SLC, Inc., (Shizuoka, Japan) and were fed an American Institute of Nutrition (AIN)-93G-based diet [21] for a one-week acclimation period. Each rat was individually housed in a separate cage with free access to food and water, except on the days preceding the glucose tolerance test and euthanasia. The experiment was performed in a temperature-controlled room maintained at 22 °C ± 2 °C with a 12 h light/12 h dark cycle (08:00–20:00 light period). The Hokkaido University Animal Committee approved the study, and all animals were handled in accordance with the Hokkaido University guidelines for the care and use of laboratory animals. After the acclimation period, rats were fed an HFS diet (30% fat and 40% sucrose, wt/wt) [8,20] for a total of 7 weeks *ad libitum* and were used for experiments after overnight fasting.

2.2. Oral glucose tolerance test (OGTT)

An OGTT was conducted after rats were fed an HFS diet for 6 weeks. Rats were fasted overnight (16–18 h) and baseline (fasting) blood was collected twice (at −15 min and 0 min) from the tail vein. Following baseline (0 min) blood collection, a glucose solution was orally administered at 2 g/kg containing 0.5% hydroxypropyl methylcellulose (10 mL/kg). Canagliflozin, added to the glucose solution, was administered at 3 or 10 mg/kg. Systemic blood was collected from the tail vein into tubes containing heparin (final concentration, 50 IU/mL; Ajinomoto Company, Inc., Tokyo, Japan), aprotinin [500 kallikrein inhibitor (KI) units/mL; Wako Pure Chemical Industries, Ltd., Osaka, Japan], and the DPP-IV inhibitor (50 μM; DPP4-010; Merck Millipore Co., Billerica, MA) at 0, 15, 30, 60, 90, and 120 min after the administration of glucose.

Plasma was separated by centrifugation at 2300 × *g* for 10 minutes at 4 °C and frozen at −80 °C until glucose, insulin, GLP-1, and GIP were measured. Plasma glucose, insulin, and total GLP-1 concentrations were measured using Glucose CII test (Wako), rat insulin enzyme-linked immunosorbent assay (ELISA; AKRIN-010T; Shibayagi Co., Ltd., Gunma, Japan), multi-species GLP-1 total ELISA (EZGLP1T-36K; Merck Millipore, Darmstadt, Germany), and rat/mouse GIP (total) ELISA (EZRMGIP-55K, Merck Millipore) kits, respectively.

2.3. Portal blood collection after the oral administration of canagliflozin with glucose

After a 7-week feeding period, a glucose solution (2 g/kg) containing canagliflozin (3 or 10 mg/kg) was orally administered to fasted (16–18 h) rats. Blood samples were collected 15 min later from the portal vein into a syringe containing heparin (final concentration, 50 IU/mL), aprotinin (500 KIU/mL), and the DPP-IV inhibitor (50 μM; DPP4-010; Merck Millipore), under sodium pentobarbital anesthesia (50 mg/kg, Somnopentyl injection; Kyoritsu Seiyaku Corporation, Tokyo, Japan). Plasma was collected and stored as described above to measure active GLP-1 and GIP levels using GLP-1 (active) ELISA (EGLP-35K; Merck Millipore) and rat GIP (Active) ELISA (YK251, Yanaihara Institute, Shizuoka, Japan) kits, respectively, in addition to the measurement of total GLP-1 and GIP levels.

2.4. GLP-1 secretion study in GLP-1-producing cells

GLUTag cells (provided by Dr. D. J. Drucker, University of Toronto, Canada), a GLP-1 producing murine cell line, were cultured in Dulbecco's modified Eagle medium (DMEM; Invitrogen, cat. no. 12100–038) containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin [22]. Cells were initially seeded in a 48-well culture plates at 1.25 × 10^5 cells/well and were grown for 2 days until they reached 80%–90% confluency. Cells were washed twice with HEPES buffer (NaCl, 140 mM; KCl, 4.5 mM; HEPES, 20 mM; $CaCl_2$, 1.2 mM; $MgCl_2$, 1.2 mM,; and BSA, 1 mg/mL; pH 7.4, without D-glucose) to remove the culture medium and were then exposed to various concentrations of canagliflozin (0.1–30 μM), which were dissolved in the same buffer supplemented with 0.1% dimethylsulfoxide for 60 min at 37 °C. HEPES buffer containing 70 mM potassium chloride (KCl) was used as the positive control. Supernatant collected from the wells after exposure was centrifuged at 800 × *g* for 5 min at 4 °C to remove remaining cells and stored at −50 °C until total GLP-1 levels was measured using the ELISA kit as described above.

2.5. Statistical analyses

Data are expressed as mean ± the standard error of the mean (SEM). Statistical analyses were performed using the JMP Pro version 12.0 software (SAS Institute, Inc., Cary, NC). Statistical significance was assessed using one-way or two-way analysis of variance (ANOVA) with time and treatments and their interaction. Significant differences ($P < 0.05$) compared to the control treatment or baseline (0 min) values were determined using the Dunnett's test.

3. Results

3.1. Effect of co-administration of glucose and canagliflozin on plasma GLP-1, GIP, insulin, and glucose levels assessed during OGTT in rats fed an HFS diet for 6 weeks

Rats were divided into three groups according to their body weight on the day of OGTT (control group, 444.5 ± 15.2 g; 3 mg/kg canagliflozin group, 442.4 ± 13.4 g; and 10 mg/kg canagliflozin group, 444.3 ± 11.8 g). Levels immediately before glucose administration (baseline) of plasma glucose, insulin, GLP-1, and GIP slightly, but not significantly, differed between the treatment groups (Supplementary Fig. 1); hence, the results are presented as changes (Δ) from the baseline values.

After the oral administration of glucose, plasma glucose and insulin levels were increased in each treatment group (Fig. 1A and B). Decreased glycemic response was observed after the administration of glucose with 10 mg/kg of canagliflozin than that of glucose alone, although the plasma glucose level at each time point did not significantly differ between the treatment groups (Fig. 1A). Two-way ANOVA revealed a significant effect of canagliflozin ($P = 0.012$). The plasma insulin level at 30 min after the