TITLE

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

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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 using 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 at 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 decrease 15 minutes 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 portion 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.

Keywords

diet–induced obesity, canagliflozin, glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, sodium-glucose cotransporter 1(SGLT1), SGLT2 inhibitor

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

Conflict of Interest

This work was supported by Mitsubishi Tanabe Pharma Corporation. S. K. is an employee of Mitsubishi Tanabe Pharma Corporation. No other authors have any conflict of interest regarding the present study.

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].

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 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 GLUTag cells, which is a murine GLP-1 producing enteroendocrine cell line.

MATERIALS AND METHODS

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 hour light/12 hour 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.

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 hours) and baseline (fasting) blood was collected twice (at −15 minutes and 0 minutes) from the tail vein. Following baseline (0 minutes) 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 minutes after the administration of glucose.

Plasma was separated by centrifugation at 2,300 × *g* for 10 minites 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.

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 and 10 mg/kg) was orally administered to fasted (16–18 hours) rats. Blood samples were collected 15 minutes 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 μmol/L; 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.

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 minutes 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 minutes 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.

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 ($P < 0.05$) between the control treatment and baseline (0 minutes) values were determined using the Dunnett's test.

RESULTS

Effect of co-administration of glucose and canagliflozin on plasma GLP-1, GIP, insulin, and glucose levels assessed using 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 Figure 1); hence, the results are presented as changes (Δ) from the baseline values.

After the oral administration of glucose, plasma glucose and insulin levels increased in each treatment group (Fig. 1A, 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 minutes after the administration of glucose was significantly lower in the 10 mg/kg canagliflozin group than in the control group (Fig. 1B), whereas the incremental area under the curve (AUC) of plasma insulin levels did not significantly differ between the groups (Fig. 1F). Plasma GLP-1 levels in the 3 mg/kg and 10 mg/kg canagliflozin groups significantly increased at 15 minutes after the administration of glucose and then declined to baseline levels (Fig. 1C). In contrast, GLP-1 levels in the control group did not significantly increase from baseline levels and were lower after 60 minutes. There were significant differences in GLP-1 levels between the 10 mg/kg canagliflozin group and the control group at 15, 60, 90, and 120 minutes. The incremental AUC of GLP-1 was significantly

higher in the 10 mg/kg canagliflozin group than in the control group (Fig. 1G). Plasma GIP levels increased after the administration of glucose in all treatment groups (Fig. 1D), whereas the levels were significantly lower at 15 and 30 minutes after the administration of glucose in the 10 mg/kg canagliflozin group than in the control group. Although GIP level was higher in the 10 mg/kg canagliflozin groups at 90 minutes after the administration of glucose than in the control group, there was no significant difference in AUC between any groups.

Effect of co-administration of glucose and canagliflozin on plasma GLP-1 and GIP levels in the portal vein of rats fed an HFS diet for 7 weeks.

Rats were maintained on an HFS diet for 7 weeks with a mean final body weight of 461.4 ± 7.4 g. To confirm the effect of canagliflozin on GLP-1 secretion from enteroendocrine L cells, blood was collected from the portal vein at 15 minutes after the oral administration of glucose with or without canagliflozin. Both total and active GLP-1 levels were significantly higher in the 3 mg/kg and 10 mg/kg canagliflozin groups than in the control group (Fig. 2A, B). Plasma total and active GIP levels trended to be lower in the 10 mg/kg canagliflozin group than in the control group; however the difference was not statistically significant (Fig. 2C, D).

Effect of canagliflozin on GLP-1 secretion in a GLP-1-producing enteroendocrine cell line.

To examine the direct effect of canagliflozin on GLP-1 secretion by enteroendocrine cells, murine GLUTag cells were exposed to canagliflozin (0.1–30 μM); no increase in GLP-1 secretion was observed in those cells (Fig. 3).

DISCUSSION

The present study demonstrated increased GLP-1 levels and decreased GIP levels after the administration if canagliflozin in obese animals, as assessed using OGTT. GLP-1 exerts regulatory effects on plasma glucose levels and is involved in control of appetite. Further, GLP-1 receptor agonists, exenatide and liraglutide, have been shown to promote weight loss in obese subjects [1-3]. In contract, GIP may be involved in the development of obesity [3, 4] because deletion of GIP receptor in mice has been demonstrated to alleviate obesity under HFD conditions. Obesity is a major risk factor for various diseases, including T2DM and cardiovascular diseases. Although a positive correlation generally exists between GIP and GLP-1 secretion after the oral administration of glucose [23], differential effects of canagliflozin on GLP-1 and GIP secretion may have benefits in the prevention or improvement of obesity.

The early response of plasma GIP in the systemic circulation to glucose loading was suppressed by the concomitant administration of canagliflozin. Intragastric administration of glucose results in its absorption by SGLT1 and stimulates GIP secretion from K cells predominantly located in the duodenum and jejunum [13-15]. Lower levels of active and total GIP in the portal vein at 15 minutes after the administration of canagliflozin and glucose than after the administration of glucose alone were attributable to a suppressed secretion rather than enhanced degradation of GIP. Glucose excursions trended to be suppressed by canagliflozin in the first 30–60 minutes after the administration of glucose. Although there was no difference in total glucose absorption between control and canagliflozin-treated groups, it has been reported that glucose absorption is delayed because of transient inhibition of SGLT1 in

the upper intestine in diabetic animals [16]. Because GIP response rapidly changed after the oral administration of glucose and canagliflozin inhibited GIP secretion when simultaneously administered with glucose, it is likely that canagliflozin lowers glucose transport via the inhibition of SGLT1 to reduce GIP secretion in the duodenum. Fasting and postprandial GIP levels are often increased rather than decreased in obese subjects [24] and genetic elimination of GIP receptors prevents the development of obesity in high-fat-diet-fed rodents [4]. Therefore, the effect of canagliflozin in decreasing GIP levels may be advantageous to promote weight loss.

In contrast to plasma levels of GIP, plasma total GLP-1 levels were higher in the 10 mg/kg canagliflozin group than in the control group. Furthermore, increases in both total and active GLP-1 levels in the portal blood were higher at 15 minutes after the administration of canagliflozin with glucose. Canagliflozin reportedly has not effect on DPP-4 activity [17]. Therefore, the increase in GLP-1 levels by canagliflozin is thought to be because of increased GLP-1 secretion rather than reduced GLP-1 degradation. GLP-1 is released from enteroendocrine cells in response to nutrients and non-nutrients [22, 25-27], including isomaltulose, a slowly digestible disaccharide [5], a dietary peptide [6,7] and a prebiotic fiber [8]. Although, canagliflozin is a glucoside compound, it did not affect spontaneous GLP-1 secretion from cultured GLUTag cells. Therefore, increased plasma GLP-1 levels are unlikely to be due to the direct action of canagliflozin on GLP-1 secretion from enteroendocrine cells.

Plasma glucose response to the administration of glucose tended to be slightly lower in the first 30 minutes in the canagliflozin group than in the control group, whereas there was no difference in the overall glucose levels. It has been shown that intragastric administration of canagliflozin inhibits carbohydrate absorption in the upper small

intestine and increases the concentration of carbohydrates in the lower small intestine in rats [16]. Consistent with the previous results, the time course of glucose excursions indicates that glucose absorption is not totally suppressed after the administration of canagliflozin. Blocking the SGLT1 activity may attenuate postprandial glucose excursions by reducing intestinal glucose absorption and may deliver unabsorbed glucose to the lower portion of the small intestine. GLP-1-producing L cells are not only present in the jejunum but also abundantly expressed in the ileum and the large intestine [6, 28]. It is likely that the increased glucose delivery into the middle or lower portion of the small intestine induced an enhanced GLP-1 response from L cells.

Canagliflozin reduces the body weight in obese patients with T2DM [10]. The primary mechanism underlying weight reduction is the extensive caloric loss into the urine through the inhibition of SGLT2 and extensive glucose excretion under hyperglycemic conditions [9-12]. The suppression of glucose-induced GIP secretion observed in obese models is likely to be mediated by the inhibition of SGLT1 and thus be independent of plasma glucose levels. The results of the present study indicate that transient inhibition of SGLT1 may provide beneficial effects on the prevention or improvement of obesity through differential regulation of GLP-1 and GIP secretion. Further, dietary factors sharing similar activity as that of canagliflozin may have a potential in controlling endogenous incretin levels in obesity.

REFERENCES

[1] D.J. Drucker, Deciphering metabolic messages from the gut drives therapeutic innovation: the 2014 Banting Lecture, Diabetes, 64 (2015) 317-326.

[2] J.J. Holst, Incretin hormones and the satiation signal, Int J Obes (Lond), 37 (2013) 1161-1168.

[3] D.J. Drucker, The biology of incretin hormones, Cell Metab, 3 (2006) 153-165.

[4] K. Miyawaki, Y. Yamada, N. Ban, et al., Inhibition of gastric inhibitory polypeptide signaling prevents obesity, Nat Med, 8 (2002) 738-742.

[5] T. Hira, M. Muramatsu, M. Okuno, et al., GLP-1 secretion in response to oral and luminal palatinose (isomaltulose) in rats, J Nutr Sci Vitaminol (Tokyo), 57 (2011) 30-35.

[6] T. Hira, T. Mochida, K. Miyashita, et al., GLP-1 secretion is enhanced directly in the ileum but indirectly in the duodenum by a newly identified potent stimulator, zein hydrolysate, in rats, Am J Physiol Gastrointest Liver Physiol, 297 (2009) G663-671.

[7] T. Mochida, T. Hira, H. Hara, The corn protein, zein hydrolysate, administered into the ileum attenuates hyperglycemia via its dual action on glucagon-like peptide-1 secretion and dipeptidyl peptidase-IV activity in rats, Endocrinology, 151 (2010) 3095-3104.

[8] T. Hira, R. Suto, Y. Kishimoto, et al., Resistant maltodextrin or fructooligosaccharides promotes GLP-1 production in male rats fed a high-fat and high-sucrose diet, and partially reduces energy intake and adiposity, Eur J Nutr, (2017) DOI. 10.1007/s00394-00017-01381-00397.

[9] M.A. Nauck, Update on developments with SGLT2 inhibitors in the management of type 2 diabetes, Drug Des Devel Ther, 8 (2014) 1335-1380.

[10] N. Rosenthal, G. Meininger, K. Ways, et al., Canagliflozin: a sodium glucose co-transporter 2 inhibitor for the treatment of type 2 diabetes mellitus, Ann N Y Acad Sci, 1358 (2015) 28-43.

[11] R. Grempler, L. Thomas, M. Eckhardt, et al., Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors, Diabetes Obes Metab, 14 (2012) 83-90.

[12] Y. Liang, K. Arakawa, K. Ueta, et al., Effect of canagliflozin on renal threshold for glucose, glycemia, and body weight in normal and diabetic animal models, PLoS One, 7 (2012) e30555.

[13] V. Gorboulev, A. Schurmann, V. Vallon, et al., Na(+)-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion, Diabetes, 61 (2012) 187-196.

[14] H.E. Parker, A.M. Habib, G.J. Rogers, et al., Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells, Diabetologia, 52 (2009) 289-298.

[15] E.M. Wright, D.D. Loo, B.A. Hirayama, Biology of human sodium glucose transporters, Physiol Rev, 91 (2011) 733-794.

[16] T. Oguma, K. Nakayama, C. Kuriyama, et al., Intestinal Sodium Glucose Cotransporter 1 Inhibition Enhances Glucagon-Like Peptide-1 Secretion in Normal and Diabetic Rodents, J Pharmacol Exp Ther, 354 (2015) 279-289.

[17] T. Oguma, C. Kuriyama, K. Nakayama, et al., The effect of combined treatment with canagliflozin and teneligliptin on glucose intolerance in Zucker diabetic fatty rats, J Pharmacol Sci, 127 (2015) 456-461.

[18] T. Oguma, C. Kuriyama, K. Nakayama, et al., Changes in glucose-induced plasma active glucagon-like peptide-1 levels by co-administration of sodium-glucose cotransporter inhibitors with dipeptidyl peptidase-4 inhibitors in rodents, J Pharmacol Sci, 132 (2016) 255-261.

[19] D. Polidori, S. Sha, S. Mudaliar, et al., Canagliflozin lowers postprandial glucose and insulin by delaying intestinal glucose absorption in addition to increasing urinary glucose excretion: results of a randomized, placebo-controlled study, Diabetes Care, 36 (2013) 2154-2161.

[20] S. Nakajima, T. Hira, H. Hara, Postprandial glucagon-like peptide-1 secretion is increased during the progression of glucose intolerance and obesity in high-fat/high-sucrose diet-fed rats, Br J Nutr, 113 (2015) 1477-1488.

[21] P.G. Reeves, Components of the AIN-93 diets as improvements in the AIN-76A diet, J Nutr, 127 (1997) 838s-841s.

[22] M. Kato, T. Tani, N. Terahara, et al., The Anthocyanin Delphinidin 3-Rutinoside Stimulates Glucagon-Like Peptide-1 Secretion in Murine GLUTag Cell Line via the Ca2+/Calmodulin-Dependent Kinase II Pathway, PLoS One, 10 (2015) e0126157.

[23] K. Mortensen, L.L. Christensen, J.J. Holst, et al., GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine, Regul Pept, 114 (2003) 189-196.

[24] T. Vilsboll, T. Krarup, J. Sonne, et al., Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus, J Clin Endocrinol Metab, 88 (2003) 2706-2713.

[25] D.Y. Guo, D.W. Li, M.M. Ning, et al., Yhhu4488, a novel GPR40 agonist, promotes GLP-1 secretion and exerts anti-diabetic effect in rodent models, Biochem Biophys Res Commun, 466 (2015) 740-747.

[26] H. Pham, H. Hui, S. Morvaridi, et al., A bitter pill for type 2 diabetes? The activation of bitter taste receptor TAS2R38 can stimulate GLP-1 release from enteroendocrine L-cells, Biochem Biophys Res Commun, 475 (2016) 295-300.

[27] D. Fukuoka, F. Okahara, K. Hashizume, et al., Triterpene alcohols and sterols from

rice bran lower postprandial glucose-dependent insulinotropic polypeptide release and prevent diet-induced obesity in mice, J Appl Physiol (1985), 117 (2014) 1337-1348.

[28] B. Svendsen, J. Pedersen, N.J. Albrechtsen, et al., An analysis of cosecretion and coexpression of gut hormones from male rat proximal and distal small intestine, Endocrinology, 156 (2015) 847-857.

FIGURE Legends

Fig. 1. Changes in plasma glucose, insulin, GLP-1, and GIP levels after the oral administration of glucose with or without canagliflozin.

OGTT was conducted in rats maintained on an HFS diet for 6 weeks and fasted overnight. After baseline blood collections (–15 min and 0 min) from the tail vein, a glucose solution (2 g/kg) with or without canagliflozin (3 or 10 mg/kg) was orally administered at 0 minutes, and blood samples were further collected until 120 min. Plasma glucose (A), insulin (B), total GLP-1 (C), and total GIP (D) levels are shown as changes (Δ) from the baseline levels (at 0 minutes). The AUC of incremental glucose (E), insulin (F), GLP-1 (G), and GIP (H) were calculated using the trapezoidal rule. Values are represented as mean ± SE (n = 7–8). P-values obtained using repeated two-way ANOVA for time were all <0.0001; for treatment, they were 0.012 in glucose, 0.0034 in insulin, <0.0001 in GLP-1, and 0.1816 in GIP; for time × treatment, they were 0.5973 in glucose, 0.0055 in insulin, 0.2315 in GLP-1, and <0.0001 in GIP (A–D). In panels A–D, asterisks (*) indicate $P < 0.05$ vs the control (glucose alone) treatment at the same time point (Dunnett's test). In panel C, plus (+) signs indicate $P < 0.05$ vs the baseline level (at 0 minutes) in each group (Dunnett's test). In panels E-H, asterisks (*) indicate $P < 0.05$ vs the control treatment (Dunnett's test).

Fig. 2. Portal GLP-1 and GIP levels 15 minutes after the oral administration of glucose with or without canagliflozin.

A glucose solution (2 g/kg) with or without canagliflozin (3 or 10 mg/kg) was orally administered to rats fasted overnight after being fed an HFS diet for 7 weeks. Blood was

collected from the portal vein after 15 minutes under anesthesia. Values are represented as mean ± SE (n = 7–8). Asterisks (*) indicate $P < 0.05$ vs control (glucose alone) treatment (Dunnett's test).

Fig. 3. Effects of canagliflozin on GLP-1 secretion in GLUTag cells.

GLUTag cells were exposed to various concentrations of canagliflozin (0.1–30 μM) or 70 mM KCl for 60 min. Supernatants were collected and GLP-1 contents were assayed using ELISA. Values are represented as mean ± SE (n = 3–4). Asterisks (*) indicate $P < 0.05$ vs the blank treatment (Dunnett's test).

Supplementary Fig. 1. Plasma glucose, insulin, GLP-1, and GIP levels after the oral administration of glucose with or without canagliflozin.

OGTT was conducted in rats fasted overnight after being fed an HFS diet for 6 weeks. A glucose solution (Glc) with or without canagliflozin (3 or 10 mg/kg) was orally administered at 0 minutes, and blood samples were collected from the tail vein. Values are represented as mean ± SE (n = 7–8). P-values obtained using repeated two-way ANOVA for time were all <0.0001; for treatment, they were 0.0166 in glucose, 0.0006 in insulin, 0.0343 in GLP-1, and 0.0044 in GIP; for time × treatment, they were 0.8546 in glucose, 0.0079 in insulin, 0.5271 in GLP-1, and <0.0001 in GIP. Asterisks (*) indicate $P < 0.05$ vs the control (glucose alone) treatment at the same time point (Dunnett's test). In panel C; plus (+) signs indicate $P < 0.05$ vs the baseline (0 minutes) level in each group (Dunnett's test).

Figure 1

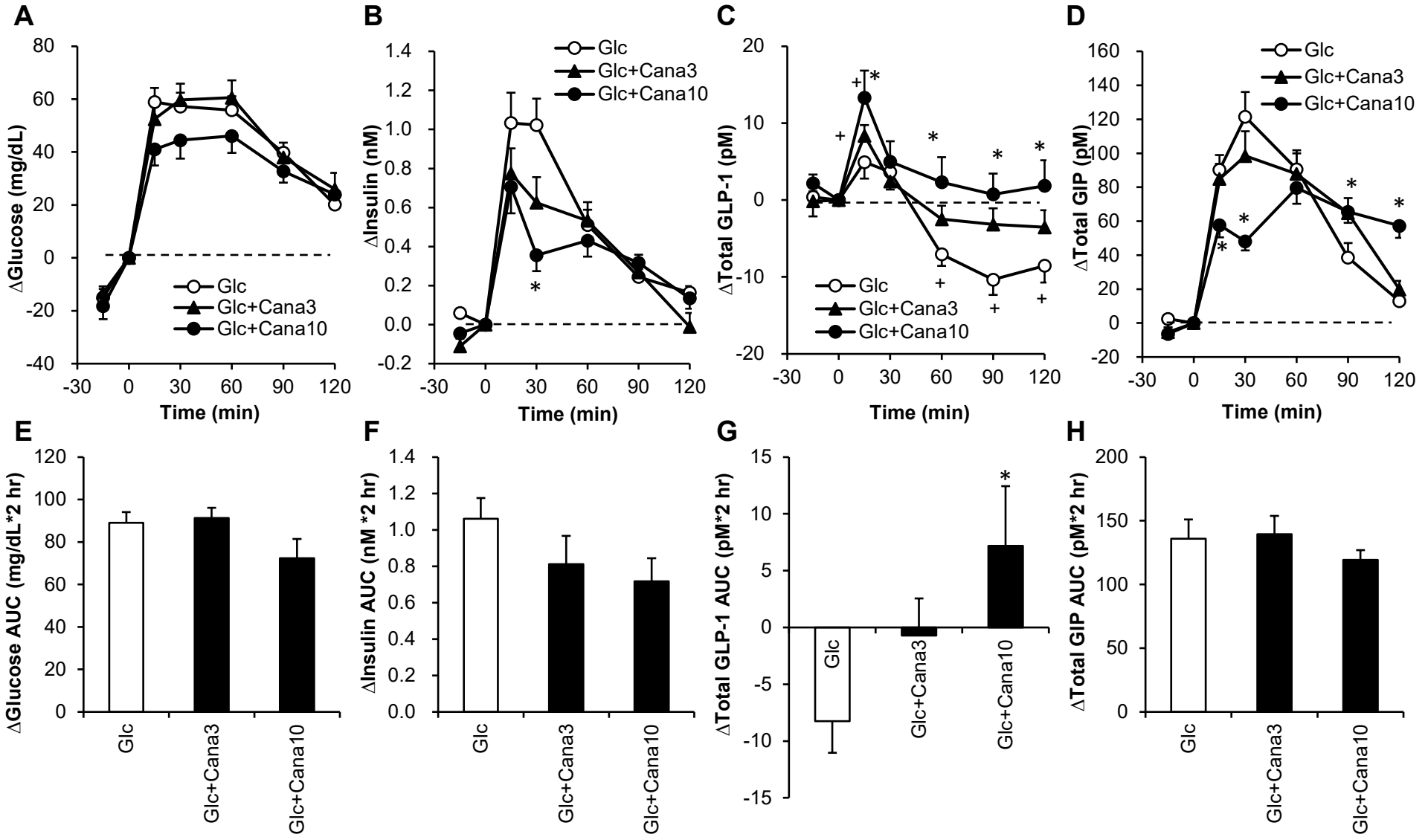
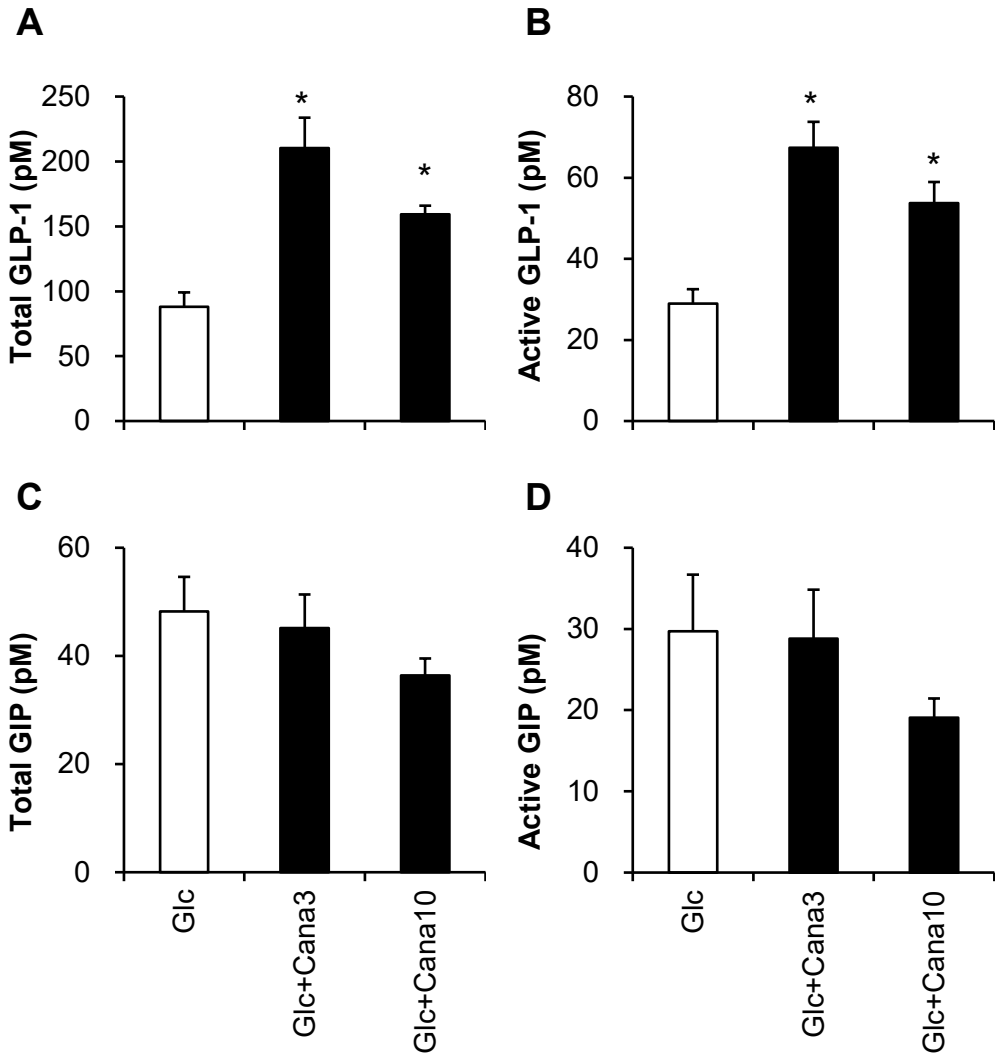

A
ΔGlucose (mg/dL)
Time (min)
Glc
Glc+Cana3
Glc+Cana10
B
ΔInsulin (nM)
Time (min)
C
ΔTotal GLP-1 (pM)
Time (min)
D
ΔTotal GIP (pM)
Time (min)
E
ΔGlucose AUC (mg/dL*2 hr)
F
ΔInsulin AUC (nM *2 hr)
G
ΔTotal GLP-1 AUC (pM*2 hr)
H
ΔTotal GIP AUC (pM*2 hr)

Figure 2


A
B
C
D
Total GLP-1 (pM)
Active GLP-1 (pM)
Total GIP (pM)
Active GIP (pM)
250
200
150
100
50
0
80
60
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Glc
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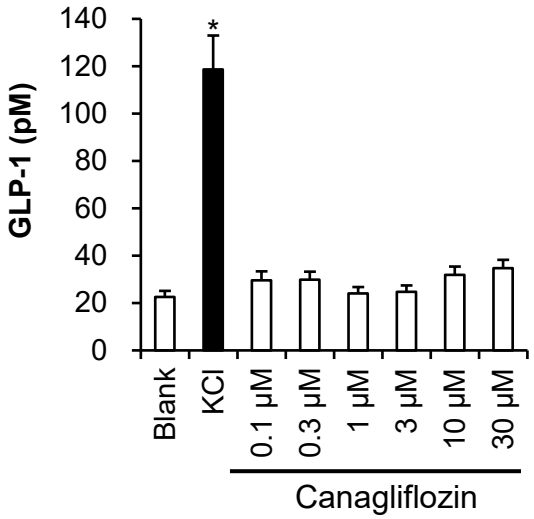
Glc
Glc+Cana3
Glc+Cana10

Figure 3


GLP-1 (pM)
140
120
100
80
60
40
20
0
*
Blank
KCl
0.1 μM
0.3 μM
1 μM
3 μM
10 μM
30 μM
Canagliflozin

Graphical abstract