Title

GLP-1 secretion in response to oral and luminal palatinose (isomaltulose) in rats

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Palatinose induces GLP-1 secretion in rats

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「ラットにおけるパラチノースの経口投与、腸管内投与に対する GLP-1 分泌応答」

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＜要約＞

消化管ホルモン Glucagon-like peptide-1 (GLP-1)は、糖質や脂質、ペプチドの摂取により、下部消化管に多く分布する GLP-1 産生細胞より分泌される。消化速度の遅い二糖類パラチノース（isomaltulose）が、GLP-1 の分泌を促進するどうか、またその機構について、ラットおよび GLP-1 産生細胞株を用いて検討した。

SD 系雄性ラットにおいて、パラチノースまたはスクロース溶液を、無麻酔下で経口投与、または麻酔下で空腸、回腸に直接投与し、門脈血中のグルコース、GLP-1 濃度を経時的に測定した。また、マウス由来 GLP-1 産生細胞株をパラチノース溶液に暴露し、上清中の GLP-1 濃度を測定した。

覚醒ラットにおいて、パラチノース経口投与群の血中 GLP-1 濃度は、スクロース投与群よりも高値を示した。麻酔下ラットにおいては、パラチノース、スクロースともに回腸投与の方が空腸投与よりも強く GLP-1 分泌を誘導した。血中グルコース濃度は、いずれの試験においてもパラチノース投与群が低値を示した。グルコースは GLP-1 産生細胞株からの GLP-1 分泌を促進したが、パラチノース、スクロースは促進しなかった。以上より、パラチノースがラット腸管において GLP-1 分泌を刺激することが見出され、その作用はパラチノースより遊離したグルコースへの寄与が大きいことが示唆された。

Summary

Palatinose (isomaltulose), a slowly digested disaccharide, is used as a non-cariogenic sugar and as a sucrose substitute in several foods. Because of its ability to lower postprandial glycemia, palatinose may be beneficial as a treatment for impaired glucose metabolism. Glucagon-like peptide-1 (GLP-1) improves glycemia via enhancing pancreatic beta-cell functions. The secretion of GLP-1 is stimulated by sugars, including glucose and artificial sweeteners. In this study, we examined whether palatinose induced GLP-1 secretion *in vivo* and *in vitro*. Firstly, portal GLP-1 and glucose were measured after oral administration of palatinose or sucrose in conscious rats. Secondly, portal GLP-1 and glucose were measured after jejunal or ileal administration of each sugar in anesthetized rats. Finally, GLUTag, a murine GLP-1-producing cell line, was exposed to several sugars, including palatinose and sucrose, to observe the direct effect of these sugars on GLP-1 secretion. Compared with sucrose, palatinose enhanced portal GLP-1 level when administered orally in conscious rats. Both palatinose and sucrose induced a significant increase in portal GLP-1 after jejunal or ileal administration of each sugar in anesthetized rats. Ileal administration triggered a greater response than did jejunal administration. Glycemic responses were higher in sucrose-treated rats than in palatinose-treated rats in every experiment. In GLUTag cells, glucose induced a significant increase in GLP-1 secretion, but neither sucrose nor palatinose had an effect. These data demonstrate that luminal palatinose induces GLP-1 secretion in rat. However, it is likely that GLP-1 secretion is triggered mainly by glucose released in the lumen rather than by palatinose itself.

Key words

Palatinose, Isomaltulose, GLP-1, enteroendocrine cells

Introduction

Achieving glycemic control is critical for the prevention and treatment of diabetes and other disorders of glucose metabolism. Inhibitors of α-amylase or α-glucosidase are used to prevent postprandial hyperglycemia by delaying carbohydrate metabolism and thereby reducing glucose absorption from the gut. Delaying carbohydrate metabolism also enhances the secretion of a gut hormone, glucagon-like peptide-1 (GLP-1) *(1)*. When intact carbohydrates reach the distal small intestine, they are slowly digested there, and glucose is released into the lumen. The middle and distal regions of the small intestine contain 'L-type' enteroendocrine cells that produce GLP-1, glucagon-like peptide-2, and/or peptide-YY *(2)*. Luminal glucose is a potent stimulator of GLP-1 secretion from L cells *(3,4)*.

GLP-1 has several biological functions: it protects pancreatic β-cells, and it enhances β-cell proliferation and the release of insulin. GLP-1 attenuates hyperglycemia acutely via insulin release; in addition, long-term treatment with GLP-1 or its analogue improves insulin-sensitivity in animal models and in human subjects *(5-8)*. Given the functions of GLP-1 and the location of the cells that secrete it, it is important to note that luminal glucose in the distal intestine, but not in the proximal small intestine, can be beneficial for glycemic control.

Palatinose (6-O-D-glucopyranosyl-D-fructose, or isomaltulose) is a disaccharide that consists of glucose and fructose connected through an α-1,6-glucosidic bond. Sucrose consists of glucose and fructose connected through an α-1,2-glucosidic bond. Unlike sucrose, palatinose is slowly hydrolyzed by brush-border isomaltase *(9,10)*, however, it is completely digested and absorbed as monosaccharides (glucose and fructose) in the small intestine *(11)*. Several papers have demonstrated that palatinose treatment is effective in preventing postprandial hyperglycemia and in improving insulin sensitivity *(12-16)*. These effects are primarily explained by the slower release and absorption of glucose. It has also been demonstrated that palatinose itself inhibits glucose absorption in an everted intestinal

sac model *(17)*. Although both palatinose and GLP-1 have beneficial effects on glycemia, the effect of palatinose on GLP-1 secretion is unknown.

In the present study we examined whether oral and luminal palatinose induce GLP-1 secretion in rats, and we determined whether palatinose itself directly stimulates GLP-1 secretion from GLP-1-producing enteroendocrine cells.

Materials and Methods

Animals

Male Sprague-Dawley rats (7 weeks old) weighing 210–230 g were purchased from Japan SLC (Hamamatsu, Japan). Rats had free access to water and to a semi-purified diet containing 25% casein based on AIN-93G *(18)*. They were housed in individual cages in a temperature-controlled room maintained at 23 ± 2°C with a 12-h light–dark cycle (0800–2000, light period). The study was approved by the Hokkaido University Animal Committee, and the rats were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals.

Experiment 1~ Effects of oral administration of palatinose or sucrose on GLP-1 secretion and glycemia in conscious rats.

Rats were anesthetized with sodium pentobarbital (40 mg/kg body weight, Nembutal Injection, Dainippon Sumitomo Pharma, Osaka, Japan) and subjected to a laparotomy. A small tip (7-8 mm) of a polyethylene catheter (SP 28; I.D. 0.4 mm, O.D. 0.8 mm; Natsume Seisakusyo, Tokyo, Japan) connected to a silicone catheter (Silascon No.00, I.D. 0.5 mm, O.D. 1.0 mm; Dow Corning Co., Kanagawa, Japan) was inserted into the portal vein. The catheter was prefilled with saline containing heparin (50 IU/ml; Ajinomoto, Tokyo, Japan). The free end of the portal catheter was exteriorized dorsally, which allowed the collection of portal blood under unanesthetized and unrestrained conditions. Rats had a 2-day recovery

period before the onset of the experiment. The portal catheter was flushed daily with heparinized saline to maintain patency.

After an overnight fast, rats received an oral dose of palatinose solution (4 g/kg body weight, Mitsui Sugar Co., Ltd., Tokyo, Japan) or sucrose solution (4 g/kg body weight) via a feeding tube (Safeed feeding tube Fr. 5, TERUMO. Co., Tokyo, Japan) inserted directly into the stomach. The dose of test sugars at 4 g/kg was comparable to the dose of glucose that is widely used in the oral glucose tolerance test (2 g/kg). Blood samples (100 µl) were drawn into a syringe containing EDTA (final concentration 1 mg/ml), aprotinin (final concentration 500 kIU/ml, Wako Pure Chemical Industries, Ltd, Osaka, Japan) and Diprotin A, a dipeptidyl peptidase IV inhibitor (final concentration 100 µM, PEPTIDE Institute Inc., Osaka, Japan) through the portal catheter before (0 min) and after (15, 30, 60, 90, 120, 150, 180, 210 and 240 min) the sample administration. Plasma was separated from blood samples by centrifugation at 2,500 x g for 15 min at 4°C and was then frozen at –80°C until GLP-1 and glucose were measured. The plasma concentration of GLP-1 and insulin were measured by the GLP-1(Active) ELISA kit (Shibayagi Co. Ltd., Gunnma, Japan) and by rat insulin ELISA kit (U-type, Shibayagi Co. Ltd.), respectively, and plasma glucose was measured by the Glucose CII-test kit (Wako).

Experiment 2~ Effects of luminal administration of palatinose or sucrose on GLP-1 secretion and glycemia in anesthetized rats.

After an overnight fast, rats were anesthetized with ketamine (80 mg/kg body weight, Ketaral, Daiichi Sankyo, Tokyo, Japan) mixed with xylazine (12 mg/kg, Sigma). A catheter was inserted into the portal vein, as described above. The jejunal ligated loop, including the duodenum and jejunum, was prepared between the end of the pylorus and 45 cm distal to the ligament of Treitz. The ileal ligated loop (~45 cm) was prepared between a section 45 cm distal to the ligament of Treitz and the terminal ileum. The proximal and distal ends of

the loop were ligated with a silk thread. After basal (0 min) blood collection, a palatinose or sucrose solution (2 g/ 4 ml/ kg) was directly administered into the loop. The dose was set at half of the oral administration described above. Portal blood was collected through the portal catheter at 15, 30, 60, 90 and 120 min after administering the solutions. During the experiment, additional ketamine (40 mg/kg) mixed with xylazine (6 mg/kg) was injected to keep the rats anesthetized, and body temperature was maintained using a heating pad. Plasma GLP-1 and glucose were measured as described above.

Experiment 3~ Effects of palatinose, sucrose and various sugars on GLP-1 secretion in a GLP-1-producing enteroendocrine cell line.

GLUTag cells (a gift from Dr. D. J. Drucker, University of Toronto, Toronto, Canada), a murine GLP-1-producing enteroendocrine cell line, were grown in Dulbecco's modified Eagle's medium (Invitrogen, Cat. No. 12100-038) supplemented with 10% fetal bovine serum, 50 IU/ml penicillin, and 500 μg/ml streptomycin in a humidified 5% CO_2 atmosphere at 37°C. Cells were routinely subcultured by trypsinization after reaching 80–90% confluency. GLUTag cells were grown in 48-well culture plates at a density of 1.25 x 10^5 cells/well for 2-3 days until they reached 80-90% confluency. Cells were washed twice with Hepes buffer (140 mM NaCl, 4.5 mM KCl, 20 mM Hepes, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 0.1% bovine serum albumin, pH 7.4) to remove the culture media and were then exposed to test agents dissolved in the same buffer for 60 min at 37°C. Based on the results in previous papers *(19、20)*, all sugars were used at 20 mM (in Hepes buffer). A 70 mM KCl solution (74.5 mM NaCl, 70 mM KCl, 20 mM Hepes, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 0.1 % bovine serum albumin, pH 7.4) was used as a depolarization stimulus. Supernatants were collected from the wells, centrifuged at 800 x g for 5 min at 4°C to remove remaining cells, and then stored at –50°C until the GLP-1 concentration was measured with a commercial enzyme-immunoassay (EIA) kit (Yanaihara Institute Inc.,

Shizuoka, Japan).

Statistical analysis

Results are expressed as means ± SEM. Statistical significance was assessed using one-way or two-way ANOVA, and significant differences among mean values were determined by Student's t-test or Fisher's LSD test ($P < 0.05$).

Results

Portal blood samples were collected through the catheter before and after the intragastric administration of a palatinose or sucrose solution in conscious rats. After the sucrose administration, the plasma GLP-1 concentration (Fig. 1A) was not significantly changed throughout the experimental period (0-240 min). GLP-1 levels in palatinose-treated rats were elevated at all times except for 150 min after treatment. Although statistically significant differences between treatments at each time point were not detected, two-way ANOVA indicated a significant difference in plasma GLP-1 between palatinose-treated rats and sucrose-treated rats. The area under the curve (AUC) of plasma GLP-1 in palatinose-treated rats (34.5 ± 10.3 nM*4hr) was around 1.5-times higher than that of sucrose-treated rats (20.1 ±1.7 nM*4hr), but statistically significant difference was not detected.

Plasma glucose (Fig. 1B) was immediately increased 15 min after intragastric administration of sucrose. The glucose level peaked at approximately 200 mg/dl 15 to 30 min after the administration of sucrose and then gradually decreased. In contrast, glucose levels remained below 160 mg/dl throughout the experimental period in the palatinose-treated rats. Plasma glucose levels in palatinose-treated rats were considerably lower than those in sucrose-treated rats from 15 to 60 min; conversely, plasma glucose levels in palatinose-treated rats were slightly higher than those in sucrose-treated rats at

180 and 210 min. Changes in plasma insulin in sucrose-treated rats (Fig. 1C) were almost similar to those in plasma glucose (Fig. 1B). Insulin levels were gradually increased until 210 min in palatinose-treated rats. Peak insulin levels were similar between two treatments. Fig. 1

Plasma GLP-1 levels (Fig. 2A) gradually increased until 90 min after either the jejunal or ileal administration of test sugars in anesthetized rats. In the jejunum, the administration of sucrose and palatinose induced similar increases in portal GLP-1 for 90 min, but whereas the palatinose infusion continued to enhance portal GLP-1, the sucrose infusion did not. GLP-1 levels at 90 min after the administration of either sugar and at 120 min after the administration of palatinose were significantly higher than basal levels. In the ileum, both sugars caused a higher increase in portal GLP-1 than they did in the jejunum. GLP-1 levels at 60, 90, 120 min in sucrose-treated rats were significantly higher than the basal level, and the levels at 90 and 120 min in palatinose-treated rats were also significantly higher than the basal level. Increments of GLP-1 in palatinose-treated rats were relatively small compared with those in sucrose-treated rats. Fig. 2

Basal glucose levels were 200-250 mg/dl in all treatments (Fig. 2B) and exceeded the basal values in conscious rats (Fig. 1B). Previous reports have attributed this effect to anesthesia *(21,22)*. Indeed, glucose levels in the mesenteric vein were also ~200 mg/dl in our preliminary study, which used the same anesthetic conditions as the present study. However, changes in the glucose level in the portal vein seem to reflect glucose absorption appropriately in the present study because ketamine-induced hyperglycemia is explained by the inhibitory effect of the anesthetic on glucose-induced insulin secretion *(22)*. This inhibition might be also responsible for lower basal GLP-1 in anesthetized rats than conscious rats since insulin reportedly induces GLP-1 secretion *(23)*. Plasma glucose increased greatly after the jejunal administration of sucrose, and values from 30 to 120 min were significantly higher than the basal value. Jejunal palatinose induced a gradual

increase in plasma glucose, which reached statistical significance at 90 and 120 min. Increments were smaller than those in sucrose-treated rats. Ileal infusion of sucrose or palatinose induced gradual and similar increases in portal glucose. Increments induced by each sugar in the ileum were smaller than those induced in the jejunum. Fig. 3

To see the direct effect of different sugars on GLP-1-producing cells, GLUTag cells were exposed to palatinose, sucrose, and other saccharides. Glucose, sucralose, and a depolarization stimulus (70 mM KCl) induced a 2- to 2.5-fold increase in GLP-1 secretion; this increase confirms that GLUTag cells are susceptible to physiological stimuli, including sugars *(19,20)*. Sucrose, palatinose, and fructose, all at the same concentration as glucose (20 mM), did not trigger an increase in GLP-1 secretion in GLUTag cells.

Discussion

Palatinose is known as one of the slowly digested sugars *(9,11)*. Several studies have demonstrated its usefulness for glycemic control in animals and humans *(12-16)*. GLP-1 has also gained popularity as a means for preventing and treating glycemic disorders, including type-2 diabetes. Its therapeutic value results from its protective and proliferative effects on pancreatic β-cells and its ability to stimulate insulin secretion. GLP-1 secretion is triggered by luminal glucose *(4,19)* and sweeteners *(20)* via sugar sensors on GLP-1-producing enteroendocrine cells.

Palatinose is slowly digested so that glucose is liberated in the ileal lumen. Palatinose is as sweet as glucose and about half as sweet as sucrose *(11)*. Its sweetness level and its ability to generate glucose suggest that palatinose may stimulate GLP-1 secretion. No published reports have investigated GLP-1 secretion in response to palatinose. In the present study, we examined whether palatinose induces GLP-1 secretion *in vivo* (rats) and *in vitro* (GLP-1-producing enteroendocrine cells).

We found that portal GLP-1 levels were higher in rats treated with palatinose than in those

256 received sucrose (Fig. 1A). Although increases in GLP-1 after oral administration of
257 palatinose in conscious rats were statistically insignificant, *in situ* experiment (Fig. 2A)
258 revealed that luminal palatinose induced GLP-1 secretion in rats. In contrast, oral sucrose
259 did not enhance portal GLP-1 levels (Fig. 1A). Because direct administration of sucrose
260 into the jejunum or ileum induced a significant increase in GLP-1 (Fig. 2A), the failure of
261 oral sucrose to increase GLP-1 might be due to an insufficient delivery of sucrose into the
262 middle and distal intestinal lumen to trigger GLP-1 secretion from L cells. Sucrose passed
263 through the stomach can be easily hydrolyzed by brush-border sucrase and immediately
264 absorbed in the upper small intestine. In contrast, palatinose is slowly digested by
265 brush-border isomaltase so that it can reach the middle and distal small intestine.
266 Therefore, it is likely that palatinose itself or glucose liberated from palatinose directly
267 stimulated GLP-1 secretion from L cells. Previous studies using α-glucosidase inhibitors
268 have also suggested that a delayed release of glucose in the distal small intestine
269 enhances GLP-1 secretion *(1,3,24)*. Our *in vitro* study using murine GLP-1-producing
270 GLUTag cells supports the hypothesis that liberated glucose is responsible for stimulating
271 GLP-1 secretion because neither palatinose nor sucrose had an effect (Fig. 3).

272 Portal glucose levels were lower in palatinose-treated rats than in sucrose-treated rats in
273 the first hour of the experiment (Fig. 1B). This reflects the lower digestibility of palatinose
274 than sucrose. Sustained glucose levels in palatinose-treated rats also indicate that the
275 sugar is slowly digested and absorbed not only in the proximal small intestine but also in
276 the distal region. Insulin responses showed almost similar pattern to glucose responses in
277 each treatment (Fig. 1C). However, differences in insulin level between two treatments are
278 smaller than those in glucose level. Higher GLP-1 levels after palatiose administration
279 might be responsible for enhanced insulin responses.

280 In the ligated loop experiment, both of the test sugars (at the half dose of the oral
281 administration) induced a higher GLP-1 secretion in the ileum than in the jejunum (Fig. 2A).

This result is due to the distribution of GLP-1 producing cells in the intestine; the number of GLP-1-producing cells is higher in distal intestine than in the proximal intestine *(2,25)*. Higher GLP-1 secretion by ileal sucrose than ileal palatinose might result from the difference in the digestibility of these sugars and from the lower degree of glucose transport in the ileum than in the jejunum. Sucrose is digested more rapidly than palatinose, but the rate of glucose absorption was similar in both treatments (Fig. 2B). Therefore, it is likely that sucrose treatment caused more glucose to accumulate in the ileal lumen, thereby inducing a higher level of GLP-1 secretion than palatinose. The difference in GLP-1 secretion between conscious rats and anesthetized rats suggests that the delivery of sugars into the intestinal lumen (mediated by gastric emptying) is an important factor for stimulating GLP-1 secretion *in vivo*.

Recent papers report that the sweet-taste receptor (T1R2/3) is involved in sugar-induced GLP-1 secretion *(20,26,27)*. In the present study, glucose and sucralose (both at 20 mM) induced significant GLP-1 secretion in GLUTag cells; however, sucrose, palatinose, and fructose (all at 20 mM) did not (Fig. 3). Based on these results, glucose, as a product of the digestion of sucrose or palatinose, may mainly be responsible for stimulating GLP-1 secretion *in vivo*. A previous paper (28) demonstrated fructose-induced GLP-1 secretion in GLUTag cells with less potency than glucose, suggesting that higher concentration than 20 mM is necessary for fructose to induce significant increase in GLP-1 secretion in our experimental conditions. In case of disaccharides such as sucrose and palatinose, degradation to monosaccharide might be important to stimulate GLP-1 secretion. However, slight increases in GLP-1 by fructose and palatinose (Fig. 3) also suggest possible involvement of these components in palatinose-induced GLP-1 secretion in the intestine. Further examinations are necessary to elucidate the hypothesis.

In summary, portal GLP-1 concentrations after oral administration of palatinose were higher compared to those after sucrose administration in conscious rats. Both palatinose

and sucrose directly administered into the ileum induced a higher GLP-1 secretion than did their administration into the jejunum. Portal glucose concentrations were lower in palatinose-treated rats than in sucrose-treated rats. In GLP-1 producing cells, glucose, but not palatinose or sucrose, induced significant GLP-1 secretion. These results suggest that glucose released from palatinose in the ileum is mainly responsible for stimulating GLP-1-producing cells to secrete GLP-1.

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403

403 **Figure legends**

404 Fig. 1. GLP-1, glucose and insulin concentrations in the portal vein after intragastric
405 administration of palatinose or sucrose in conscious rats.

406 Palatinose (4 g/kg, closed circle) or sucrose (4 g/kg, open circle) was orally administered
407 into the stomach of conscious rats. Blood samples were collected through the portal vein
408 catheter before (0 min) and after the oral sugar administration. Values are means ± SEM of
409 7-8 rats in each group. Two-way ANOVA P values for GLP-1 (A) were 0.99 for time, 0.043
410 for treatment, and 0.93 for treatment x time. The values for glucose (B) were all <0.01. The
411 values for insulin (C) were 0.02 for time, 0.69 for treatment, and 0.38 for treatment x time.
412 Asterisk (*) signs indicate significant differences between treatments (Student's t-test, $P <$
413 0.05).

414

415 Fig. 2. Plasma GLP-1 and glucose after the instillation of a palatinose or sucrose solution in
416 the ligated jejunal or ileal loop in anesthetized rats.

417 Palatinose (Pal: 2 g/kg, closed circle) or sucrose (Suc: 2 g/kg, open circle) was directly
418 administered into the jejunum (Jej: thinner line) or ileum (Ile: thicker line) of anesthetized
419 rats. Blood samples were collected through the portal vein catheter before (0 min) and after
420 the sugar administration. Values are means ± SEM of 7-8 rats in each group. Two-way
421 ANOVA P values for GLP-1 (A) were < 0.01 for time and for treatment, and 0.898 for
422 treatment x time. Two-way ANOVA P values for glucose (B) were < 0.01 for time and for
423 treatment, and 0.549 for treatment x time. Plots with a plus (+) sign differ significantly from
424 the value at 0 min in each group (Fisher's test, $P < 0.05$). Plots at the same time point not
425 sharing the same letter (a, ab, b) differ significantly between treatments (Fisher's test, $P <$
426 0.05).

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Fig. 3 GLP-1 secretion in response to various sugars in GLUTag cells

GLUTag cells were exposed to various sugars (20 mM), a sweetener (sucralose at 20 mM) or 70 mM KCl for 60 min. The supernatant was collected and the GLP-1 concentration was measured with GLP-1 EIA. Values are relative (%) to control GLP-1 secretion and expressed as means ± SEM (n = 6-8). Values not sharing the same letter differ significantly ($P < 0.05$ by Fisher's test).

436 **FIGURE 1**

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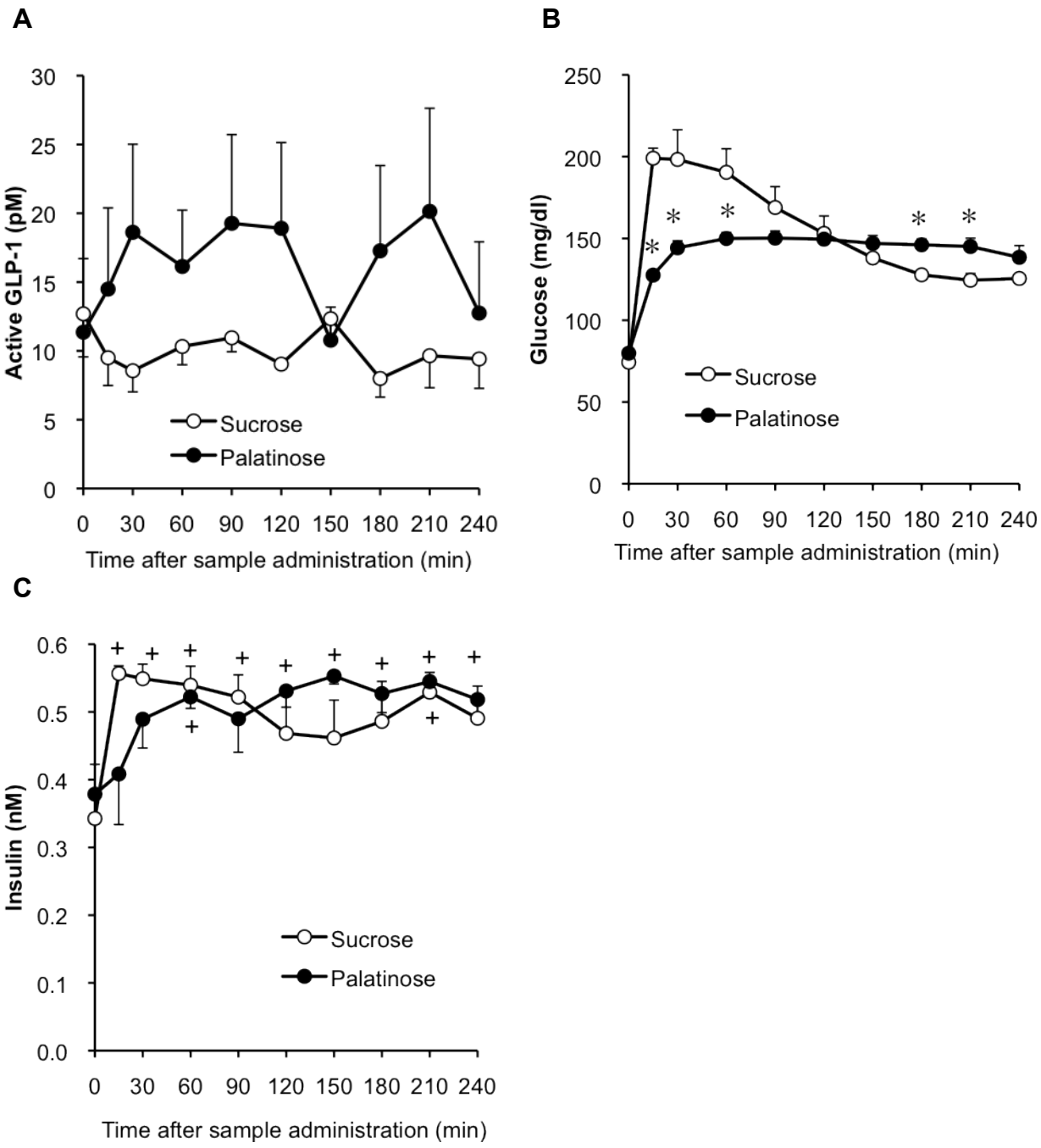

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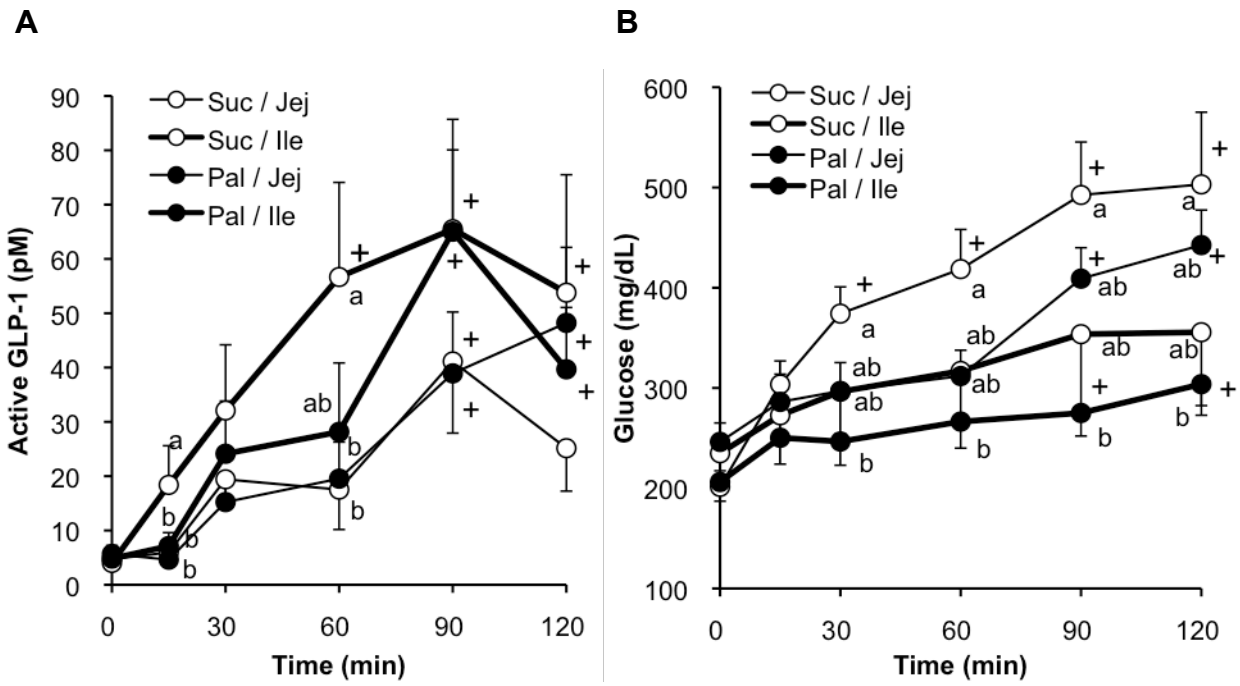
441 **Hira, Figure 1**

442

FIGURE 2



Hira, Figure 2

449 **FIGURE 3**

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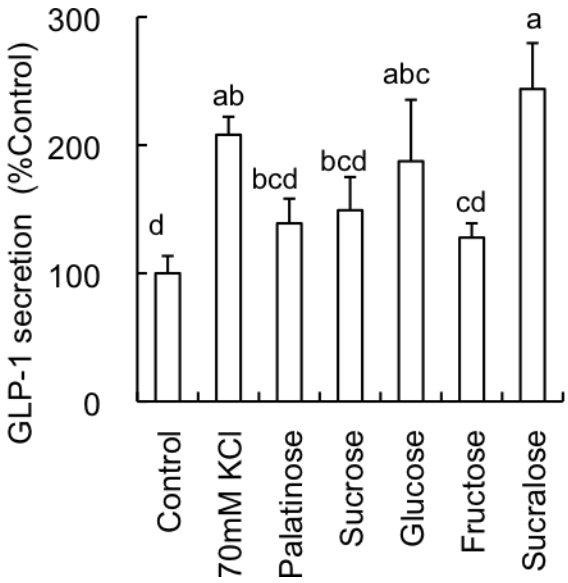

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455 **Hira, Figure 3**

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