RAPID COMMUNICATION

Dose-dependent effect of glucose on GLP-1 secretion involves sweet taste receptor in isolated perfused rat ileum

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Abstract. Luminal glucose is an important stimulus for glucagon-like peptide 1 (GLP-1) secretion from intestinal endocrine cells. However, the effects of luminal glucose concentration on GLP-1 secretion remain unknown. In this study, we investigated the effect of luminal glucose concentrations (3.5, 5, 10, 15, and 20 mmol/L) on GLP-1 secretion from isolated perfused rat ileum. Results showed that the perfusate glucose concentration dose-dependently stimulated GLP-1 secretion from isolated perfused rat ileum, which was eliminated by the sweet taste receptor inhibitor gurmarin (30 µg/mL), but not inhibited by phloridzin (1 mmol/L), a Na⁺-coupled glucose transporters inhibitor. We conclude that luminal glucose induced GLP-1 secretion from perfused rat ileum in a concentration-dependent manner. This secretion was mediated by sweet taste receptor transducing signal for GLP-1 release on the gut of rat.

Key words: Sweet taste receptor, Glucagon-like peptide 1, Glucose concentration, Rat, Ileum

FOOD INTAKE results in the secretion of incretin hormones in the body, among which glucagon-like peptide 1 (GLP-1) and glucose-dependent insulin-releasing polypeptide (GIP) are responsible for 50%–70% insulin secretion after a meal [1]. Oral administration of glucose and fat results in two overlapping phases of GLP-1 secretion [2]. The early phase begins within minutes of a meal and continues for 30–60 min. The second phase causes prolonged secretion of GLP-1 at 1–3 h after a meal. This delayed phase of secretion involves the direct detection of luminal contents through enteroendocrine L-cells [1].

Dietary nutrients, including carbohydrates, lipids, amino acids, and bile acids, can trigger GLP-1 release from particular intestinal endocrine L-cells. Specifically, glucose is a well-established stimulus for GLP-1 and GIP secretion *in vivo*. Studies suggested

that luminal glucose in the proximal gut directly stimulates GLP-1 secretion. In addition, luminal glucose administration acutely stimulates GLP-1 secretion from isolated perfused mouse intestines [3]. Similar results were obtained when glucose was perfused into segments of the small intestines of rats, dogs, and pigs, in which luminal glucose infusion stimulates GLP-1 secretion [4]. The stimulating secretion mechanism of luminal glucose has been extensively investigated. Studies using the GLUTag cell line showed that L cells may use the glucose-sensing machinery involving glucose phosphorylation, enhanced glycolytic and mitochondrial metabolism, and closure of ATP-sensitive potassium channels [5], the membrane depolarizes, the opening of voltage gated calcium channels, the generation of Ca²⁺-carrying action potentials, and stimulation of exocytosis [6]. *In vivo* studies suggested that luminal glucose stimulation of GLP-1 secretion also results from the activity of Na⁺-coupled glucose transporters (SGLTs) [7, 8]. These concomitantly carry Na⁺ ions for each glucose molecule transported, thereby generating small depolarizing currents sufficient to trigger electrical activity and Ca²⁺ entry and consequent increased GLP-1 secretion from L-cell [8]. In recent years,

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some data provide extensive evidence for the involvement of a-gustducin-coupled sweet taste receptors in glucose-stimulated GLP-1 secretion in human and rodent gut [9]. Sweet taste receptor comprises a heterodimer of the G protein-coupled type 1 taste receptors TAS1R2 and TAS1R3, which activates downstream signaling pathways involving a-gustducin (GNAT3), phospholipase C β 2, and the transient receptor potential cation channel TRPM5 [10]. However, our previous experiments on the effect of non-systemic glucose administration on blood glucose revealed that chronic drinking of glucose solution does not increase serum GLP-1 levels [11]. We deduced that GLP-1 secretion from intestinal L-cells is related to luminal glucose concentration and that this secretion involves a certain glucose-sensing pathway. Therefore, isolated perfused rat ileum was investigated in the present study.

Materials and Methods

Animals

Twenty-four male Sprague–Dawley rats (200–250 g) were obtained from the Department of Laboratory Animal Science, Peking University Medical College, China. The rats were maintained in an air-conditioned room at $23 \pm 1^\circ\text{C}$ and 50–60% relative humidity, with a 12 h light–dark regime. They were provided with a regular laboratory diet and water *ad libitum*. All experimental procedures were approved by the Ethics Committee on Animal Experiment of Hebei North University and conformed to the Office Regulations of China. All efforts were performed to ameliorate suffering of animals.

Rat ileum perfusion experiments

This study was carried out by referring to Hansen's and Ritzel's method of small intestinal perfusion [12, 13]. The rats were used for experiments after overnight fasting but allowed with water *ad libitum*. The animals were anesthetized *via* intraperitoneal injection of sodium pentobarbital (1%, 50 mg/kg). Laparotomy was performed through a midline incision. Ileum segments (≈ 10 cm), including their arterial and venous supplies, were isolated, excised, and submerged in Ringer's solution. After rat ileum segments were obtained, the rats were euthanized *via* an intracardiac injection of sodium pentobarbital. Catheters were inserted into an artery and a vein. The arterial catheter was attached to a perfusion pump, and the venous cath-

eter was used to collect venous effluent. It was perfused in a single-pass system using a gassed (5% CO $_2$ in O $_2$) Krebs–Ringer–bicarbonate solution. The perfusion solution contained 0.1% human serum albumin (Sigma–Aldrich), 5% dextran T-70 (Sigma–Aldrich), amino acid mixture (14 g/L Vamin (Pharmacia), a total concentration of 5 mmol/L, glucose (5 mmol/L), insulin 1 nM (Sigma–Aldrich), and 20% freshly washed bovine erythrocytes. The perfusion flow rate was 0.2–0.3 mL/g/min and remained constant. The intestinal lumens were inserted with a catheter. The upper end of the catheter was attached to a perfusion pump and perfused with oxygenated, preheated perfusate (containing different glucose concentrations, 5% dextran T-70, and 0.1% human serum albumin at a flow rate of 2 mL/min). A circulator bath was used to maintain the steady temperature of the perfusion system at 37°C throughout the experiment.

Arterial catheter attached to perfusion pump in intestinal lumens is exactly working due to failure to detect haemoglobin in luminal effluent, no net water flux across intestinal wall and at joint between arterial and catheter were observed during perfusion.

Experimental protocol

A total of 24 perfusion experiments were carried out. In eight experiments, we investigated the effect of luminal glucose on GLP-1 release at different concentrations. In eight experiments, perfusion medium containing different glucose concentrations was added with phloridzin (1 mmol/L, Sigma–Aldrich). In eight experiments, perfusion medium was added with gurmardin (30 $\mu\text{g/mL}$, Sigma–Aldrich). In each experiment, perfusion was conducted for 60 min, including a 30 min equilibration period. The venous effluent was collected at 5 min intervals, centrifuged (1,088 $\times$ g/min, 5 min at 4°C), and immediately frozen before further analysis. Luminal effluent after glucose perfusion was collected for glucose determination.

GLP-1, dipeptidyl peptidase-4 (DPP-4), and glucose analysis

The GLP-1 concentrations of the venous effluent were analyzed using a commercially available enzyme-linked immunosorbent assay kit (Epitope Diagnostics, Inc.), according to the instructions of the manufacturer.

DPP-4 activity in the venous effluent was also measured using a commercial assay kit (Sigma–Aldrich).

The standard curve was generated using 4-nitroaniline. Enzymatic activities were measured spectrophotometrically at 405 nm.

Moreover, glucose concentrations of luminal effluent after glucose perfusion were determined by using a commercial assay kit (BioSino, China). Glucose absorption rates were also calculated.

Calculations

Results are presented as the mean $\pm$ SEM ($n=8$). Statistical differences between groups were analyzed *via* Student's *t*-test or ANOVA, followed with Tukey's multiple comparison test where appropriate. All statistical calculations were performed using SPSS 17.0 software, and differences resulting in *p*-values of less than 0.05 were considered statistically significant.

Results

Effect of glucose concentrations on GLP-1 release

Following luminal perfusion with different glucose concentrations (30–60 min), GLP-1 levels in the venous effluent were measured, and the results are shown as the area under GLP-1 concentration–time curve for 30–60 min. Glucose perfusions with 3.5 mmol/L concentration exerted no significant effect on GLP-1 release,

whereas perfusion with 5, 10, 15, and 20 mmol/L luminal glucose concentration dependently influenced the amount of GLP-1 release (Fig. 1). Groups of 5, 10, 15, and 20 mmol/L glucose perfusion showed significant difference for GLP-1 release ($p<0.05$) *via* ANOVA and Tukey's multiple comparison analysis.

Effect of phloridzin on GLP-1 release

The addition of phloridzin (1 mmol/L), an inhibitor of SGLTs receptor in GLP-1-producing cells and glucose transport of lumene, in perfusion did not inhibit GLP-1 release caused by luminal glucose perfusion (Fig. 1).

Effect of gurmarin on GLP-1 release

In perfusion of each glucose concentration, gurmarin (30 μ g/mL), a sweet taste receptor inhibitor in rodents, was added. GLP-1 secretion in response to a range of glucose concentrations in the presence and absence of gurmarin was analyzed. The results showed that gurmarin eliminated luminal perfusate glucose-stimulated GLP-1 secretion from isolated perfused rat ileum (Fig. 2). Statistical difference was observed for the groups of 10, 15, and 20 mmol/L glucose perfusion with gurmarin, compared with that without gurmarin ($p<0.01$).

Fig. 1 GLP-1 release in the venous effluent and glucose concentrations in ileum perfused glucose with or without phloridzin (Phlz, 1 mmol/L)

GLP-1 release was shown as the area under the GLP-1 concentration–time curve (AUG) for 30–60 min. Significance was determined using *t* test. * $P<0.05$, among groups of 5, 10, 15, and 20 mmol/L glucose perfusion, by ANOVA followed by the Tukey's multiple comparison test.

Fig. 2 GLP-1 release in the venous effluent and glucose concentrations in ileum perfused glucose with or without gurmarin (Gur, 30 μ g/mL)

GLP-1 release was shown as the area under the GLP-1 concentration–time curve (AUG) for 30–60 min. Significance was determined using *t* test. * $P<0.05$, vs without gurmarin.

DPP-4 activity

DPP-4 activity analysis was performed in each venous effluent collected. Enzymatic activities are shown as 4-nitroaniline concentration, which is the cleavage product of Gly-Pro-*p*-nitroanilide hydrochloride. Comparisons among groups were conducted, and no significant difference for DPP-4 activity was observed among groups during perfusion of glucose in the presence and absence of phloridzin and gurmardin ($p > 0.05$, Fig. 3).

Discussion

The role of glucose as an important stimulus in the regulation of GLP-1 secretion has been widely studied in experiments involving animals with knockout genes, isolated perfused small intestine, primary cultures of enteroendocrine cells from rodent intestines, and cell lines [3, 4, 14]. In this study, the effects of different concentrations of glucose on GLP-1 secretion were observed in isolated perfused rat ileum. The results demonstrated that GLP-1 secretion in rat ileum was obviously glucose concentration-dependent, and that amount of GLP-1 release increased with perfusion glucose concentration. There were no significant change for dipeptidyl peptidase-4 (DPP-4) activity during glucose perfusion. The result coincides with Hansen's study [12], which reported that the perfusate glucose concentration (3.5, 5, and 11 mmol/L) influences the amount of GLP-1 secreted, using isolated preparation of porcine ileum. Kuhre study also

showed that Luminal glucose (5% and 20% w/v) stimulated GLP-1 secretion dose dependently [4].

The mechanism of glucose-induced GLP-1 secretion from gut involves enteric hormonal or neural signals, and direct glucose sensing by the L-cells. Some studies have shown that several direct glucose-sensing pathways have been implicated in glucose triggered GLP-1 secretion from L-cells. One pathway is associated with the classical glucose-sensing machinery employed by the pancreatic β cell, mediated through glucose metabolism and closure of ATP-sensitive K^+ channels, which are acted upon by glucose from blood [2] or might be modulated by neuronal inputs [15]. Another pathway is Na^+ -coupled glucose transporters (SGLT1) distributed on the gut luminal brush border membrane, which mediates gut luminal glucose action [8, 15]. SGLT1 transports sodium ions and glucose concomitantly and further produces electrogenic signals, which trigger glucose-induced GLP-1 secretion [16]. SGLT1 is also the main transporter of gut glucose absorption, with high affinity to glucose. When the gut glucose concentration is lower than that of plasma, SGLT1 transports glucose into enterocytes against its concentration [7]. The third pathway involves the sweet taste receptor (T1R2/T1R3), a G protein-coupled receptor (GPCR) including key elements such as α -gustducin, phospholipase $C\beta 2$, and transient receptor potential channel type 5, all of which also exist in enteroendocrine cells in the gut [17-20], α -gustducin has been detected in brush cells of rat gut [21, 22].

Fig. 3 DPP-4 activity in each venous effluent during perfusate glucose in the presence and absence of phloridzin and gurmardin
Enzymatic activities was shown as 4-nitroaniline concentration.

In the present study, gut luminal perfusions with 3.5 mmol/L glucose did not influence GLP-1 secretion. However, Reimann demonstrated that glucose might cause GLP-1 release at concentrations ranging from 0 mmol/L to 25 mmol/L using an *in vitro* culture of the murine GLUTag cell line [2]. This difference in the results from isolated perfused preparation of ileum and culture cell line may be related to the sensitivity of glucose-sensing pathways on L-cells, suggesting that glucose receptors facing the gut lumen (SGLT1 and sweet taste receptor) demonstrated lower sensitivity to luminal glucose than the receptors on the plasma membrane.

To observe the role of SGLT1 in glucose concentration-dependent GLP-1 secretion, the SGLT1 inhibitor phloridzin (1 mmol/L) was added to luminal perfusate buffer. Results showed that phloridzin did not inhibit GLP-1 release from perfusion ileum, thereby indicating that SGLT1 was not the main glucose-sensing pathway in gut and other mechanisms might more important. It is known that the impact of SGLT1 inhibition of GLP-1 levels is controversial and likely depends on the experimental model. Earlier data in isolated intestinal segments and cell cultures showed that blocking of SGLT1 suppressed GLP-1 secretion. An opposite effect was demonstrated *in vivo*. The reason that our result is different from other isolated perfusion intestine and cell cultures is not clear. It is perhaps related to populations of L cells, the concentrations of perfused glucose, segments of isolated intestine, and the segments is whether contain other enteroendocrine cells (for example, expressing sweet taste receptors) affecting GLP-1 release from L cells indirectly.

Some studies have provided evidence for the involvement of α -gustducin-coupled sweet taste receptors in glucose-stimulated peptide secretion [17, 20, 23]. It is been shown by Jang [20] that L cells of the gut sense glucose through the same mechanisms used by taste cells of the tongue. Direct sensing of glucose by taste signaling elements expressed in L cells leads to GLP-1 release from these L cells [20].

Mouse endocrine cells of the GLUTag line showed markedly increased GLP-1 and GIP secretion upon exposure to the sweetener sucralose, which was subsequently blocked by the sweet taste inhibitor gurmarin [9]. In the present study, gurmarin, a sweet taste receptor inhibitor in rodents, was added to luminal glucose perfusate to observe the role of sweet taste receptor in glucose concentration-dependent GLP-1 release.

Gurmarin, a protein extracted from *Gymnema sylvestre*, inhibits sweetener-mediated calcium responses of cells expressing the rat T1R2/T1R3 by binding sweet taste receptor protein [24]. As shown in the results, co-administration of gurmarin in glucose perfusate eliminated GLP-1 secretion induced by luminal glucose perfusate at a range of glucose concentrations. Our result suggest that sweet taste receptor has important implications for glucose concentration-dependent GLP-1 release, which are also supported by several *in vitro* and *in vivo* studies. Mice knockout models for α -gustducin (α -gust^{-/-}) or T1R3 (T1R3^{-/-}) showed deficiencies in glucose-stimulated secretion of GLP-1 [19]. Sweetener sucralose induce GLP-1 secretion from the culture of human enteroendocrine NCI-H716 cell line in a concentration-dependent manner [20]. Lactisole, a sweet taste receptor inhibitor specific to human and other primates, can inhibit the effects of sucralose-stimulated GLP-1 release from NCI-H716 cells [20], as well as block glucose-stimulated secretion of GLP-1 in humans *in vivo* [25].

However, further evidence showed that artificial sweeteners did not stimulate GLP-1 release in primary cultures, and that purified L-cells were not found to express high levels of components of the sweet-taste-receptor pathway [15]. It is been shown by Sutherland *et al.* [26] and Yonug *et al.* [27] that α -gustducin in rodents is expressed in small portion of L-cells (15%), but its density is higher in other enteroendocrine cells (brush cells 57%, 5-HT-containing enterochromaffin cells 27%). It have been shown that L cell exist distinct populations of L cells, and that glucose-sensitive L cells could be located in the upper intestine, with glucose-insensitive L cells located in the lower intestine [1]. These facts suggested that GLP-1 release from perfused rat ileum is not due to direct glucose sensing by L-cells in ileum. Therefore, the possibility remains that in the intestinal segment sweet taste receptors may affect GLP-1 release from L-cells in ileum indirectly. It is likely that other mediators released *via* α -gustducin signaling in other enteroendocrine cells take part in the regulation of GLP-1 release from L cells.

The present study shown that 5-20 mmol/L glucose increased GLP-1 secretion significantly in isolated perfused rat ileum. However, even 50 mmol/L glucose does not taste so sweet in our tongue [28]. This discordance herhaps is related to sweet sensitivity. Growing evidence shown that sweet sensitivity can be modulated by some hormone and other factors that act on

sweet-sensitive receptor cells. Orexigenic and anorexigenic factors such as endocannabinoids and leptin may affect sweet sensitivity. Endocannabinoids may enhance sweet taste responses in mice, and leptin selectively inhibit sweet taste responses by leptin receptor expressed in sweet sensitive receptor cells in mice [29, 30]. Glucagon-like peptide-1 normally acts to maintain or enhance sweet taste sensitivity by its paracrine activity [29]. However, in present study, we used isolated perfused rat ileum, which exclude the effect of endocannabinoids, leptin and some factors on sweet sensitivity. Moreover, Kitagawa *et al.* [31] study shown that the topographic distribution of T1R3 in various taste papillae was different from those of the other T1R members and that T1R3 affect sweet sensitivity of mice. Their result indicate that T1R3 may serve as the receptor for sweet perception in mice. Young [27] indicated that intestinal sweet taste molecules was dynamic regulation of expression and that levels of sweet taste molecule transcripts are modulated in the intestine by

both luminal and metabolic factors. The present study, sensitive of sweet taste receptor to glucose may also be related to the difference of T1R3 expression level in the intestine from that from tongue although we did not observe this difference.

In conclusion, the present results indicated that luminal glucose stimulated GLP-1 secretion in a concentration-dependent manner, which was dependent on the sweet taste receptor transducing signal for GLP-1 release in perfused rat ileum.

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