Relationship between molecular structures of sugars and their ability to stimulate the release of glucagon-like peptide-1 from canine ileal loops

Kenji Shima¹, Takeo Suda², Kayo Nishimoto³ and Sachiko Yoshimoto¹

Department of Laboratory Medicine¹, School of Medicine University of Tokushima, Tokushima, and Otsuka Pharmaceutical Co Ltd.², Kagasuno Tokushima, Japan

Abstract. The structure-activity relationship of sugars inducing secretion of glucagon-like peptide-1 from the gut was examined using intestinal loops prepared from the terminal portion of the ileum of dogs. The plasma glucagon-like peptide-1 concentration in a mesenteric vein draining only the looped region of the intestine was increased after infusion of 139 mmol/l solutions of D-glucose, D-galactose, D-glucuronic acid, 3-0-methyl-D-glucose, maltose, sucrose or maltitol into the intestinal lumen, but not after infusion of solutions of D-fructose, D-fucose, D-mannose, D-xylose or lactose. The increases in plasma glucagon-like peptide-1 concentration correlated with the corresponding increases in glucagon-like immunoreactivity induced by these sugars. The plasma glucose level of the regional mesenteric vein increased significantly from the basal level after instillation of D-glucose, but not after instillation of other sugars. It is suggested that cells of the gut have a glucose sensor for release of products of the glucagon gene and that this sensor has specific steric requirements. The sugars that induced glucagon-like peptide-1 release share the molecular features of electron density near C(6), an equatorial hydroxyl at C(2), and an axial hydroxyl at C(1), which could account for their recognition by the glucose sensor to initiate the releases of glucagon-like peptide-1 and glucagon-like immunoreactivity.

Gut glucagon-related peptides are distributed in enteroendocrine L cells throughout the gastrointestinal tract, with highest concentrations localized in the distal small intestine and the colon (1-3). The precursor molecule proglucagon contains, in addition to the amino acid sequence of the hormone glucagon and glicentin-related polypeptide (proglucagon(1-30)), a sequence of further 96 amino acids. The latter fragment contained two glucagon-like peptides, glucagon-like peptide (GLP)-1 (proglucagon(72-108)) and glucagon-like peptide-2 (proglucagon(126-159)), which were flanked by basic dipeptides, potential post-translational cleavage sites (4,5) Proglucagon is shown to be processed into glicentin (proglucagon 1-69) (6) and the two glucagon-like peptides in the ileum. Recently, the complete amino acid sequences of human and porcine gut GLP-1 have been shown to correspond to that of proglucagon(78-107) amide (7) which was the product of further cleavage and amidation of proglucagon(72-108). A synthetic peptide corresponding to proglucagon(78-107) amide has been reported to have several interesting effects: at physiological concentrations it strongly stimulates insulin secretion (8,9) suppresses glucagon secretion (10,11), and inhibits acid secretion (12). GLP-1 immunoreactivity has been shown to be released into the circulation after a mixed meal and in response to a glucose load (13). Thus, GLP-1 has been suggested to play an important role in carbohydrate metabolism as an "incretin". However, the mechanism of its secretion from the intestinal tract is not known.

To clarify this point with special reference to the mechanism of induction of GLP-1 release from the gut by sugars, we compared the effects of various sugars, sugar derivatives, and a sugar alcohol on...
the release of GLP-1 from ileal loops of dogs with the levels of glucagon-like immunoreactivity (GLI) release.

Materials and Methods

Ninety-eight healthy mongrel dogs of both sexes, weighing 10.5±1.7 kg (mean ± sd), were maintained on commercial dog food. Before experiments they were starved for 24 h and then anesthetized with sodium pentobarbital (30 mg/kg). A loop of the ileum was prepared by ligating both ends of a 30-cm segment of the distal portion of the ileum as described by Matsuyama et al. (14). A polyethylene tube was inserted into the loop from the proximal end for rinsing and also for introducing a solution of sugar. The externalized loop was covered with gauze moistened with 154 mmol/l NaCl solution. Test solutions of 278 mmol/l monosaccharides or their derivatives (D-forms of glucose (N=10), galactose (N=8), glucuronic acid (N=8), 3-O-methyl-glucose (N=8), fructose (N=8), fucose (N=6), mannose (N=8), and xylose (N=6) and disaccharides or their derivatives maltose (N=8), sucrose (N=8), lactose (N=7), and maltitol (N=8) were prepared one day before experiments. Just before experiments, these test solutions were mixed with an equal volume of 150 mmol/l phosphate buffer (pH 7.4) and heated to 37°C. 154 mmol/l of NaCl was used in 5 dogs as a nonsugar control agent. Observations were carried out for 15 min after a 10-min control period.

Blood was taken from a mesenteric vein that drained only the looped region of the intestine. Blood samples obtained at each time were promptly transferred to chilled tubes containing 1 g EDTA-Na2 and 5×10⁵ units of aprotinin per l of blood. The tubes were kept in ice and centrifuged as soon as possible, and the resultant plasma was stored at ~20°C until analysed.

GLP-1 was measured by radioimmunoassay using an antibody against GLP-1 (Peninsula Labs, Inc, San Carlos, CA). LMT-01 whose characteristics were reported elsewhere (15). In brief, it had no cross-reactivity with insulin, somatostatin, vasoactive intestinal polypeptide, secretin, peptide histidine-isoleucine, glucagon-like peptide-2 at their respective doses up to 2000 μg/l, but very low cross-reactivity, less than 0.001%, with glucagon. In this assay, the lowest detectable amount of GLP-1 is 12 pmol/l and the inter- and intra-assay coefficients of variation are 6 and 4.5%, respectively. GLI was measured by a double-antibody RIA with OAL-196 against the immunodeterminant to proglucagon (33-58) (16). The inhibition curve for glicentin (NOVO, Denmark) was superimposed on that for pancreatic glucagon in this assay system (16). The intra- and inter-assay coefficients of variation were 3.8 ~ 5.0 and 6.0 ~ 7.3%, respectively, in this assay system.

Glucose was measured in a Hitachi 705 autoanalyzer with a kit containing glucose oxidase and mutarotase (Wako Pure Chemical Industries Co, Osaka).

Chemicals, all of analytical grade, were obtained from the following sources: D-forms of monosaccharides, such as fructose, galactose, glucose, 3-O-methyl-glucose and xylose, and maltose, sucrose, and lactose from Wako Pure Chemical Industries, Osaka; fucose from Aldrich Chemical Co, Milwaukee, WI; maltitol from Hayashibara Chemical Co, Okayama; mannose from Tokyo Kasai Co, Tokyo.

Calculations and statistical analyses

Significance of difference in plasma immunoreactive GLP-1 and glucose concentrations from their respective basal levels was evaluated by two-way analysis of variance. Significance of correlation between increment of plasma immunoreactive GLP-1 concentration and that of GLI concentration during the observation period was analysed by Student's t-test. Data are presented as mean ± SEM.

Results

Fig. 1 shows the GLP-1 responses to various hexoses and their derivatives. The plasma GLP-1 concentration was significantly increased from basal level as early as two minutes after infusion of D-glucose, or D-glucuronic acid into the intestinal lumen and continued to increase or remained at an elevated level until the end of the experiment. After instillation of D-galactose or 3-O-methyl-D-glucose plasma GLP-1 concentration was significantly increased from basal level at several points during the observation period. No such increase in plasma GLP-1 concentration was observed after instillation of D-fructose, D-mannose or D-fucose. Fig. 2 shows the GLP-1 responses to various kinds of disaccharides, their derivatives, pentose, and saline. Maltose, sucrose and maltitol stimulated GLP-1 release from the intestine, but lactose, D-xylose and saline did not.

We examined the relationship of increment of the plasma immunoreactive GLP-1 concentration to that of GLI during instillations of the various sugars into loops of the ileum. As shown in Fig. 3, the increment of the plasma GLP-1 concentration correlated with the corresponding value of GLI to similar extents for all sugars that stimulated the secretions of the peptides (r=0.86, 0.89, 0.91, 0.89, 0.88 and 0.94 for D-glucose, D-galactose, D-glucuronic acid, 3-O-methyl-D-glucose, sucrose and maltitol, respectively), except maltose, for which the correlation coefficient was smaller (r=0.62). These findings suggest that GLP-1 and GLI were secreted...
in equimolar amounts from the gut irrespective of the kind of sugar inducing peptide secretion.

The plasma glucose level of the regional mesenteric vein increased significantly (p<0.01) from a basal level of 5.74±0.18 mmol/l to 6.63±0.26 mmol/l at 4 min after instillation of the D-glucose solution into the loop, and continued to increase thereafter, reaching a level of 7.30±0.29 mmol/l at the end of the experiment. No increase in the plasma glucose level was observed when other kinds of sugar were instilled, irrespective of whether they did or did not induce releases of GLP-1 and GLI.
Discussion

In this study, using loops of canine ileum, we examined the structure-activity relationship of sugars inducing secretion of gut GLP-1. Results showed that the sugars and their derivatives tested could be divided into two groups with respect to ability to stimulate gut GLP-1 release; the compounds capable of stimulating GLP-1 release, referred to as active sugars, were D-glucose, D-galactose, D-glucuronic acid, 3-O-methyl-D-glucose, maltose, sucrose and maltitol, whereas those not stimulating GLP-1 release, referred to as inactive sugars, were D-fructose, D-fucose, D-mannose, D-xylose and lactose. The test solutions used were 278 mmol/l of sugars and their derivatives mixed with an equal volume of 150 mmol/l phosphate buffer. We used isotonic solutions since luminal hypotonicity per se, but not hypertonicity, was reported to be one of the stimuli of gut GLI from the ileum (17). D-glucose has been reported to stimulate the secretion of GLP-1 from isolated perfused porcine ileum (18) and in man (13), and our data confirm these reports. However, there have been no reports of the abilities of other sugars to stimulate GLP-1 release from the gut. In enteroglucagon-producing cells, proglucagon is known to be processed to GLI, GLP-1 and GLP-2, which are secreted into the blood stream without further modification upon appropriate stimulation (18). We found that after instillation of any kind of active sugar into a loop of canine ileum, the rise in the regional plasma immunoreactive GLP-1 concentration was parallel with that of GLI, and the nearly equimolar amounts of immunoreactive GLP-1 and GLI were secreted. Previously we reported (19) that oral administration of D-galactose, but not D-fructose, in-
creased the plasma GLI level in man, suggesting that D-galactose, but not D-fructose, is capable of stimulating the release of products of the glucagon gene from tissue of the gut. Our present results are consistent with these previous findings. These findings were quite similar to those of Marco et al. (20) except for xylose which they found stimulated GLI release from canine intestine. This difference may be due to differences in the experimental design, that is, they administered the test solution intraduodenally in the conscious dogs and measured GLI concentration in peripheral blood.

Until now, there has been no substantial study on the question whether sugars alter GLP-1 secretion from the gut as a consequence of their metabolism or whether they may exert their effort through sugar sensors without being metabolized. However, judging from the α-anomeric specificity of gut GLI secretion (14), it seems likely that GLI-secreting cells in the ileum have sugar sensors, since the α-anomer of glucose is less well metabolized than the β-anomer, assuming that sugars are metabolized by these cells in a similar way as in pancreatic islets (21). On the same assumption, galactose and 3-O-methyl-D-glucose, which are poorly metabolized (22), are capable of stimulating GLP-1 and GLI release, whereas mannose, which is metabolized (23), is inactive. These findings support the notion that the effect of sugars on GLP-1 secreting cell function may be mediated at least in part via a glucose sensor mechanism. If this is the case, does the hypothetical sensor sense a particular molecular architecture to initiate GLI and GLP-1 release? D-glucose exists primarily as a six-member ring with a chair conformation in which the hydroxyl groups at C-2, C-3 and C-4 are in the equatorial position (Fig. 4). α-D-glucose with a hydroxyl group at C-1 in the axial position, has been reported to be more potent than β-D-glucose in stimulating GLI release, suggesting that the α-anomer is recognized better than the β-anomer. D-mannose, which does not stimulate GLI or GLP-1 release, gives further insight into the steric requirement at C-2 for recognition of a hexose, because it has an axial instead of an equatorial hydroxyl group at C-2. Thus, for recognition, the hexose must have an equatorial hydroxyl group at C-2. The inability of D-mannose to induce release of GLI or GLP-1 is in contrast to its ability to stimulate insulin release from pancreatic B-cells (24,25) and to suppress arginine-induced glucagon release from pancreatic A-cells (26). Even when the hy-

![Molecular structures of sugars.](image)

**Fig. 4.**
Molecular structures of sugars.
active: compounds capable of stimulating GLP-1 release
inactive: compounds incapable of stimulating GLP-1 release
broken line: axial bond
solid line: equatorial bond
droxyl group at C-3 is replaced by a methoxy group, as in 3-O-methyl-D-glucose, the compound can stimulate the releases of GLI and GLP-1. D-galactose, the C-4 epimer of D-glucose, has an axial hydroxyl group at C-4, but although it differs from D-glucose in the position of one hydroxyl group, it stimulates the releases of GLI and GLP-1. Therefore, the structures at C-3 and C-4 in D-glucose are not necessary for recognition by the GLI and GLP-1 release mechanism. Removal of the terminal CH₂OH group from D-glucose, as in D-xylene, resulted in loss of ability to induce the releases of GLI and GLP-1. Removal of the hydroxyl group at C-6, as in 6-deoxy-D-galactose (fucose), also resulted in inability to induce the releases of GLI and GLP-1, indicating the importance of an electron dense group at the C-6 position. This is compatible with the fact that glucuronic acid, which has an electron dense carboxyl group at C-6, stimulates the releases of GLI and GLP-1. The results of analyses of the effects of D-glucose and its analogues indicate that for recognition by cells releasing GLI and GLP-1, a hexose must have an electron density near C-6, an equatorial hydroxyl at C-2, and an axial hydroxyl at C-1. D-fructose, an inactive sugar, differs from D-glucose in its structures at C-1 and C-2. At the end of experiments, test solutions of disaccharides obtained from the looped lumen did not contain sufficient glucose to stimulate the releases of GLI and GLP-1 (data not shown). No significant increase in the plasma glucose level of the regional mesenteric vein was observed after instillation of the active disaccharide solutions into the loop. Therefore, it seems likely that the stimulatory effects of active disaccharides on the releases of GLI and GLP-1 were due to these compound per se, not to their hydrolysis products. The active disaccharides, maltose (0-α-D-glucopyranosyl-(1→4)-α-D-glucopyranoside), sucrose (0-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside and maltitol (0-α-D-glucopyranosyl(1→4)-0-D-glucitol), contain α-D-glucopyranosyl ring, unlike the inactive disaccharide lactose (0-β-D-galactopyranosyl-(1→4)-D-glucopyranoside). The presence of an α-D-glucopyranosyl ring seems necessary for recognition of disaccharides by cells releasing GLI and GLP-1. This conclusion is compatible with the fact that D-glucose shows α-anomeric stereospecificity for GLI release (14).

In conclusion, biological and structural studies suggest that cells in the gut inducing release of products of the glucagon gene from the gut have a glucose sensor with specific steric requirements. Sugars that induce the releases of GLI and GLP-1 share a common molecular architecture that could account for their recognition by this glucose sensor.

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References


18. Ørskov C, Holst JJ, Knuhtsen S, Baldissera FGA, Poulsen SS, Nielsen OV. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. Endocrinology 1986;119:1467-75.


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Dr Kenji Shima,
Department of Laboratory Medicine,
School of Medicine,
University of Tokushima,
Kuramoto-Cho,
Tokushima 770,
Japan.