Brief Report

Localization of glucagon-like peptide-2 receptor mRNA expression at different sites in the small intestine of rats

Kenji Ikeda[1,*], Michiaki Myotoku[1], Nobuo Kurokawa[2], Yoshihiko Hirotani[1]

[1] *Laboratory of Clinical Pharmaceutics, Faculty of Pharmacy, Osaka Ohtani University, Tondabayashi, Osaka, Japan;*
[2] *Department of Pharmacy, Osaka University Hospital, Suita, Osaka, Japan.*

ABSTRACT: Preproglucagon is known to be processed into glucagon-like peptide (GLP)-1, GLP-2, and glicentin in the L cells of the intestinal tract. GLP-2 has been shown to possess intestinotrophic activity in rodents. However, the ligand-binding mechanisms of GLP-2 receptor (GLP-2R) have not been extensively investigated. The present study sought to determine the localization of GLP-2R in the small intestine by analyzing GLP-2R mRNA expression using the reverse transcriptase-polymerase chain reaction (RT-PCR) method. GLP-2R mRNA expression was detected at all sites in the small intestine; its expression levels were particularly high in the jejunum. Moreover, GLP-2R mRNA expression was detected in both non-mucosal intestinal tissues and intestinal mucosa. These findings suggest that in addition to the intestinal mucosa, the functional sites of GLP-2 may be present in the non-mucosal tissues. These results imply that GLP-2 peptide acts as an intestinotrophic factor that may affect the intestines from outside the mucosa. The intestinotrophic effect of GLP-2 from outside the mucosa may be a function of the enteric neurons transmitting several growth signals to mucosal cells.

Keywords: GLP-2, GLP-2 receptor, Small intestine, Localization, Mucosa

Introduction

Preproglucagon, a precursor of enteroglucagon, is known to be processed into glucagon-like peptide (GLP)-1, GLP-2, and glicentin in the L cells of in the intestinal tract (*1*). GLP-1 regulates blood glucose via the stimulation of glucose-dependent insulin secretion, inhibition of gastric emptying, and inhibition of secretion of glucagons. Research has shown that GLP-2 has an intestinotrophic effect in rodents (*2*). Myojo *et al.* (*1*) demonstrated that glicentin has a direct trophic effect on rat small intestinal mucosa and on the IEC-6 rat small intestinal cell line, and they suggested that this peptide forms the active site of enteroglucagon. Moreover, the current authors reported that methionyl-rat glicentin has a growth-enhancing effect on the mucosa during an intestinal adaptive response following 70% resection of the rat small intestine (*3*). In the residual intestine of rats with 70% of the distal small intestine resected, furthermore, the jejunum mucosal weight increased as a result of GLP-2 treatment (*4*) and small intestinal injury following methotrexate administration (*5*).

The distribution of receptors to these peptides suggests that GLP-2, like glucagon and GLP-1, acts through a distinct and specific novel receptor expressed in its principal target tissue-the gastro intestinal tract (*6*). The novel expression sites (*e.g.*, jejunum) for both glucagon and GLP-1 receptor gene in the mouse have previously been identified (*7*).

GLP-2 is known to be an intestine-derived peptide with intestinotrophic properties that may be therapeutically useful in treating diseases characterized by intestinal damage or insufficiency (*8*). Nian *et al.* provided evidence demonstrating the divergence in the mechanisms of tissue-specific regulation of proglucagon genes in humans and rodents (*9*). Moreover, the structural changes and the ligand-binding mechanisms of GLP-2 receptor (GLP-2R) have previously been investigated (*10-13*). The present study sought to determine the localization of GLP-2R in the intestines by analyzing the GLP-2R mRNA expression of rat intestinal sites (*i.e.*, the duodenum, jejunum, ileum, and each mucosal site) by using a reverse transcriptase-polymerase chain reaction (RT-PCR) method.

Materials and Methods

Preparation of intestinal samples

All experimental procedures were conducted in

**Correspondence to:* Dr Kenji Ikeda, Laboratory of Clinical Pharmaceutics, Faculty of Pharmacy, Osaka Ohtani University, 3-11-1 Nishikiori-kita, Tondabayashi, Osaka 584-8540, Japan;
e-mail: ikedak@osaka-ohtani.ac.jp

accordance with Osaka University Medical School guidelines for the care and use of laboratory animals.

The experiments were carried out using male Wistar rats (180-200 g) (Clea Japan, Inc., Tokyo, Japan). The small intestines of the rats were removed 24 h after fasting. Each portion of intestinal tissue was divided into the following 3 sites: the duodenum (D), jejunum (J), and ileum (I). The mucosal tissue (M) at each site was then harvested and collected. Further, each site of non-mucosal tissue (NM) was divided into proximal (1), middle (2), and distal (3) sites. These 12 sites of the rat small intestine were used for the experiment described below. On preparation, these samples were immediately stored in liquid nitrogen until assay.

Semi-quantitative RT-PCR

Total RNA was isolated from rat intestinal tissues using an RNeasy® Mini Kit (Qiagen K.K., Tokyo, Japan) and identified by ultraviolet (UV) spectrophotometry. For the generation of first strand cDNA templates for RT-PCR, 3 μg of DNase I-treated total RNA from the rat intestines were reverse transcribed at 42°C for 50 min using the SuperScript™ First-Strand Synthesis System (Invitrogen Japan K.K., Tokyo, Japan). An aliquot of the first-strand reaction solution (volume, 1/20) was used as a template for PCR with KOD-plus DNA polymerase (Toyobo Co., Ltd., Osaka, Japan). PCR was performed over a range of cycles spanning the exponential phase of amplification kinetics, and linearity was shown for relative cDNA input across 2 orders of magnitude. Primer pairs selected for PCR were as follows: rat GLP-2R, 5′-GCTGGCCTCATTCTTATCC-3′ and 5′-AACAAGAAGAGTGTAAGAGCC-3′; and rat β-actin (encoded by the *ACTB* gene), 5′-ATCATGTTT GAGACCTTCAACAC-3′ and 5′-TCTGCGCAAGTT AGGTTTTGTC-3′. Samples for GLP-2R analysis were denatured at 94°C for 2 min and subjected to 34 cycles of PCR (94°C for 15 sec, 56°C for 30 sec, and 68°C for 1 min) with an additional extension at 72°C for 8 min; this resulted in the generation of a 291-base-pair (bp) product spanning the entire GLP-2R open reading frame. ACTB cDNA amplification was performed at an annealing temperature of 60°C for 24 cycles, resulting in the generation of an 825-bp product. ACTB cDNA amplification was used as an internal control to monitor the efficiency of first-strand cDNA synthesis. PCR products were separated by gel electrophoresis on a 1% agarose gel, stained with ethidium bromide, and imaged under UV light. The expression ratios of GLP-2R were normalized to that of the housekeeping gene *ACTB*.

Statistical analysis

Student's *t*-test was performed in order to calculate the statistical significance of differences among the means of mRNA expression levels normalized to that of ACTB. Data presented in the figures denote mean ± SD values of the results collected from at least 2 independent experiments.

Results and Discussion

In order to isolate an equal amount of total RNA from each site, the wet weights of the divided tissues were determined. The wet weight of jejunum was found to be the highest. The values of M and NM were 10.0 mg/cm and 63.8 mg/cm, respectively. However, the NM/M wet weight ratio of the duodenum and that of the jejunum were equal (*i.e.*, 6.4). Among the 3 intestinal sites, the NM/M wet weight ratio (7.3) of the ileum was the highest (Table 1). These results demonstrated that the rat ileum contains a smaller amount of mucosal tissue than the jejunum and duodenum. Further, these values of NM/M wet weight ratio were used to calculate the total expression at each site per length based on GLP-2R mRNA expression values.

Following the measurement of wet weight, amplification curves of rat GLP-2R and ACTB mRNA expression in RT-PCR were investigated using the total RNA from the jejunum in order to establish suitable conditions for semiquantitative detection. A linear relationship between PCR products and cycle numbers was obtained: 32-36 and 24-32 cycles of PCR for GLP-2R and ACTB, respectively. Accordingly, the conditions selected for the semiquantitative detection of GLP-2R and ACTB were 34 and 24 cycles of PCR, respectively.

Having determined the appropriate PCR conditions, the expression ratio of GLP-2R mRNA to ACTB mRNA at the 3 aforementioned sites was investigated. Each NM site of D, J, and I was measured after they were divided into proximal (1), middle (2) and distal (3) sites. GLP-2R mRNA expression was observed at all of the sites. Among these sites, however, GLP-2R mRNA expression was the highest in the jejunum NM site (Figure 1A). Additionally, among the rat intestinal tissues, the expression ratio of GLP-2R mRNA to ACTB mRNA was highest at the J-1 site and relatively lower at the ileum site (Figure 1B). The J-1 site was located in the initial 1-2 cm of the proximal jejunum. At the J-2 and J-3 sites, which were near the beginning of the ileum, the level of GLP-2R expression was lower.

Table 1. The wet weight and GLP-2R mRNA expression ratio of mucosal and non-mucosal tissues

Intestinal site	Wet weight			NM/M ratio of GLP-2R mRNA expression (mean ± SD, $n = 3$)
	Mucosal (M) (mg/cm)	Non-mucosal (NM) (mg/cm)	NM/M ratio	
Duodenum	8.7	55.3	6.4	1.29 ± 0.27
Jejunum	10.0	63.8	6.4	2.06 ± 1.00
Ileum	8.1	59.4	7.3	1.03 ± 0.05

NM/M ratio of GLP-2R mRNA expression represents the ratio of the GLP-2R mRNA expression normalized to ACTB mRNA.

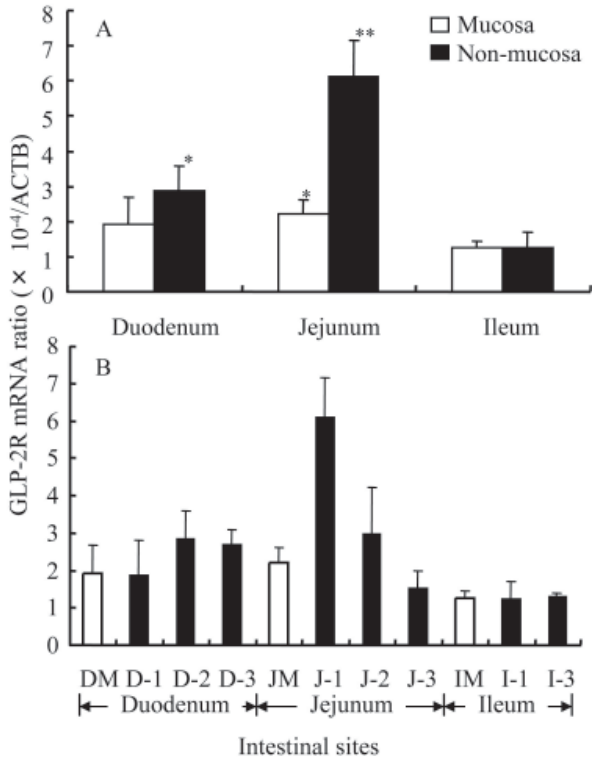

Figure 1. A: The differences in the expression ratios of GLP-2R mRNA to ACTB mRNA at intestinal sites and between mucosal (M) and non-mucosal (NM) tissues. Values are expressed as mean ± SD (n = 3). * $p < 0.05$, ** $p < 0.01$ *vs*. the ileum. B: The differences in the expression ratios of GLP-2R mRNA to ACTB mRNA among all the divided intestinal sites. Values are expressed as mean ± SD (n = 3). DM, duodenal mucosa; D-1, proximal duodenum; D-2, middle duodenum; D-3, distal duodenum; JM, jejunal mucosa; J-1, proximal jejunum; J-2, middle jejunum; J-3, distal jejunum; IM, ileal mucosa; I-1, proximal ileum; and I-3, distal ileum. GLP-2R mRNA expression was not detected in I-2, which represented the middle of the ileum.

These results suggest that GLP-2 peptide may transmit several signals to response cells at the J-1 site.

At each of the duodenum, jejunum, and ileum sites, the ratio of GLP-2R mRNA expression at the non-mucosal site to that at the mucosal site was greater than 1; this ratio was highest at the jejunum (greater than 2) (Table 1). The result indicated in Table 1 implies that at all sites the expression was not limited to the mucosa. However, these expression ratios were normalized to ACTB and the total expression levels at each site were estimated based on the wet weight of each site. This estimation indicated that total GLP-2R expression at the non-mucosal proximal jejunum site may be more than 10-fold of the level at mucosal proximal jejunum site.

GLP-2 has an intestinotrophic effect through a specific receptor expressed in its principal target tissue-the gastrointestinal tract (*14,15*). However, the cellular localization of the GLP-2 receptor and the nature of its signaling network in the gut remain poorly defined. Reports have indicated that the antiapoptotic effects of GLP-2 on intestinal crypt cells may be useful for the attenuation of chemotherapy-induced intestinal mucositis (*13,16*).

Identifying the functional site of GLP-2 peptide in the small intestine should prove beneficial to providing pharmacotherapy for several small intestinal injuries. The present study compared GLP-2R mRNA expression levels at different sites in the intestinal tract. The GLP-2R mRNA expression levels were highest in the jejunum. Further, the mRNA expression level in the jejunum, particularly in the proximal jejunum, was markedly higher at the non-mucosal site than at the mucosal site. These findings suggest that the jejunum may be the main site of GLP-2 binding and that, in addition to the intestinal mucosa, functional sites of GLP-2 may be present in the non-mucosal tissues. Additionally, GLP-2 peptide may affect the intestines as an intestinotrophic factor from outside the mucosa. Bjerknes *et al.* reported that the nervous system is a key component of a feedback loop that regulates epithelial growth and repair (*17*). The current data implicates GLP-2 as a potentially crucial neurotransmitter involved in the regulation of food intake and body weight. The intestinotrophic effect of GLP-2 from outside the mucosa may be a function of the enteric neurons, which transmit several signals to mucosal cells.

References

1. Myojo S, Tsujikawa T, Sasaki M, Fujiyama Y, Bamba T. Trophic effects of glicentin on rat small-intestinal mucosa *in vivo* and *in vitro*. J Gastroenterol 1997; 32:300-305.
2. Drucker DJ. Glucagon-like peptides. Diabetes 1998; 47:159-169.
3. Hirotani Y, Taki M, Kataoka K, Kurokawa N, Satoh T, Sasaki K, Yanaihara C, Luo WQ, Yanaihara N. Effect of rat glicentin on intestinal adaptation in small intestine-resected rats. Ann N Y Acad Sci 1998; 865:601-605.
4. Hirotani Y, Yamamoto K, Yanaihara C, Balaspiri L, Yanaihara N, Kurokawa N. Distinctive effects of glicentin, GLP-1 and GLP-2 on adaptive response to massive distal small intestine resection in rats. Ann N Y Acad Sci 2000; 921:460-463.
5. Hirotani Y, Yamamoto K, Ikeda K, Arakawa Y, Li J, Kitamura K, Kurokawa N, Tanaka K. Correlation between plasma glucagon-like peptide 2 levels and proliferative makers in small intestinal injury in rats induced by methotrexate administration. Biol Pharm Bull 2006; 29:2327-2330.
6. Munroe DG, Gupta AK, Kooshesh F, Vyas TB, Rizkalla G, Wang H, Demchyshyn L, Yang ZJ, Kamboj RK, Chen H, McCallum K, Sumner-Smith M, Drucker DJ, Crivici A. Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. Proc Natl Acad Sci USA 1999; 96:1569-1573.
7. Campos RV, Lee YC, Drucker DJ. Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. Endocrinology 1994; 134:2156-2164.
8. Drucker DJ, Boushey RP, Wang F, Hill ME, Brubaker PL, Yusta B. Biologic properties and therapeutic potential of glucagon-like peptide-2. JPEN J Parenter Enteral Nutr 1999; 23:S98-S100.
9. Nian M, Drucker DJ, Irwin D. Divergent regulation of human and rat proglucagon gene promoters *in vivo*. Am

J Physiol 1999; 277:G829-G837.
10. Yusta B, Somwar R, Wang F, Munroe D, Grinstein S, Klip A, Drucker DJ. Identification of glucagon-like peptide-2 (GLP-2)-activated signaling pathways in baby hamster kidney fibroblasts expressing the rat GLP-2 receptor. J Biol Chem 1999; 274:30459-30467.
11. DaCambra MP, Yusta B, Sumner-Smith M, Crivici A, Drucker DJ, Brubaker PL. Structural determinants for activity of glucagon-like peptide-2. Biochemistry 2000; 39:8888-8894.
12. Walsh NA, Yusta B, DaCambra MP, Anini Y, Drucker DJ, Brubaker PL. Glucagon-like peptide-2 receptor activation in the rat intestinal mucosa. Endocrinology 2003; 144:4385-4392.
13. Rocha FG, Shen KR, Jasleen J, Tavakkolizadeh A, Zinner MJ, Whang EE, Ashley SW. Glucagon-like peptide-2: divergent signaling pathways. J Surg Res 2004; 121:5-12.
14. Yusta B, Huang L, Munroe D, Wolff G, Fantaske R, Sharma S, Demchyshyn L, Asa SL, Drucker DJ. Enteroendocrine localization of GLP-2 receptor expression in humans and rodents. Gastroenterology 2000; 119:744-755.
15. Petersen YM, Elnif J, Schmidt M, Sangild PT. Glucagon-like peptide 2 enhances maltase-glucoamylase and sucrase-isomaltase gene expression and activity in parenterally fed premature neonatal piglets. Pediatr Res 2002; 52:498-503.
16. Boushey RP, Yusta B, Drucker DJ. Glucagon-like peptide (GLP)-2 reduces chemotherapy-associated mortality and enhances cell survival in cells expressing a transfected GLP-2 receptor. Cancer Res 2001; 61:687-693.
17. Bjerknes M, Cheng H. Modulation of specific intestinal epithelial progenitors by enteric neurons. Proc Natl Acad Sci USA 2001; 98:12497-12502.

(*Received December 21, 2007; Revised December 25, 2007; Accepted December 26, 2007*)