Administration of anti-glucagon-like peptide-2 serum suppresses epithelial cell proliferation of the distal small intestine in weanling rats

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Abstract

We investigated the effects of endogenous glucagon-like peptide-2 (GLP-2) on the development of intestinal mucosa in weanling rats. Three-week-old male weanling Sprague-Dawley rats were administered either anti-GLP-2 or normal rabbit serum every other day for 2 weeks. We then measured length, weight, and bromodeoxyuridine incorporation in the intestine on day 13 following the first injection. Administration of anti-GLP-2 serum significantly inhibited both epithelial proliferation in the distal ileum and elongation of the small intestine. These results suggest that intrinsic GLP-2 contributes to the growth of the small intestine during the weanling period.

Glucagon-like peptide-2 (GLP-2) is composed of 33 amino acids and is released in response to luminal nutrient stimuli from enteroendocrine L-cells (4) that reside in the distal part of the intestine (5). GLP-2 promotes epithelial proliferation and protects against intestinal inflammation in animal models (2, 8). Such intestinotropic effects have been evaluated mainly via the administration of extrinsic GLP-2 (12) or Gly-Gly-GLP-2, a GLP-2 analogue that is resistant to dipeptidyl peptidase IV (DPP-IV) (1). Transposition of a distal section of the intestine into the proximal area leads to intestinal growth. This indicates that L-cells must be exposed to a greater nutrient supply for intestinal growth to occur (13). Expression of the GLP-2 receptor and concentrations of circulating GLP-2 are enhanced during the neonatal period (9). Furthermore, the response to GLP-2 is temporarily up-regulated after birth (10). In fact, milk protein induces GLP-2 secretion in suckling rats, resulting in subsequent intestinal growth (7). GLP-2 might also play a role in intestinal development during the weanling period. In this study, we investigated whether intrinsic GLP-2 is involved in epithelial proliferation. Specifically, we blocked GLP-2 with anti-rat GLP-2 serum for a few weeks after the weanling period.

Weanling male Sprague-Dawley rats (3 weeks old; Japan SLC, Hamamatsu, Japan) weighing 40 to 60 g were used in this study. The rats were provided free access to water and standard rat chow (CE-2; CLEA Japan, Inc., Tokyo, Japan). Animals were housed in individual cages under controlled temperatures ($22 \pm 2°C$) and a 12 h light:dark cycle. This experiment was approved by the Hokkaido University Animal Use Committee, and animals were maintained according to the university's guidelines for the care and use of laboratory animals.

Antiserum against rat GLP-2 (aGLP-2; Y322, Yanaihara Institute Inc. Fujinomiya, Japan) was used to block endogenous GLP-2, and normal rabbit serum (NRS) was used as a control. Antibody fractions were purified from serum by precipitation with ammonium sulfate and reconstituted in phosphate-buffered saline (PBS). Rats (n = 12) were divided into two groups following 1 day of acclimation. Each group of rats was injected subcutaneously with either NRS or aGLP-2 (1 mg/g body weight) every other day. On day 13 after the first injection, rats

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were decapitated under diethylether anesthesia. Each rat received a subcutaneous dose of bromodeoxyuridine (BrdU) solution 1 h before sacrifice, as previously reported (7). The entire small intestine, cecum, and colorectum were removed and flushed with saline. The weight and length of the small intestine and colorectum were then measured. The small intestine was divided by length into four equal parts (duodenum, jejunum, proximal ileum, and distal ileum) that were fixed in 10% formalin in PBS for immunohistochemistry.

Frozen sections of intestinal segments were treated with 10% NRS in PBS to block non-specific binding. We used mouse anti-BrdU antibodies (Clone NA-20; Calbiochem, EMD Chemicals, Inc., Darmstadt, Germany) and biotinylated rabbit anti-mouse IgG+A+M (H+L) as the primary and secondary antibodies, respectively. We scored the epithelial cells from the bottom of the crypt to cell position 20 within a crypt under a microscope, as described previously (7). Fifty well-organized crypt sections were scored in each site of the intestine. Epithelial proliferation was evaluated as a ratio of the number of BrdU-incorporating epithelial cells to the total number of cells in the epithelial layer of the crypts.

Statistical differences between groups were assessed using a Student's *t*-test and Hsu's MCB test with JMP software (SAS institute, Cary, NC). A two-way analysis of variance was performed for quantitative data from BrdU staining (treatment × site). A *P*-value of less than 0.05 was considered statistically significant.

To investigate whether intrinsic GLP-2 influences early intestinal development in weanling rats, we blocked endogenous GLP-2 by administering aGLP-2 for approximately 2 weeks. We found that the small intestine was significantly shorter in aGLP-2-treated rats than in NRS-treated controls. A similar trend was observed in the colon; however, no significant difference was observed between treatments (Table 1). BrdU histochemistry revealed that aGLP-2 administration significantly reduced the proportion of BrdU-incorporating crypt epithelial cells in the distal ileum (Fig. 1). A two-way analysis of variance showed that aGLP-2 treatment significantly inhibited BrdU incorporation in the small intestine ($P = 0.0379$ for treatment). We did not measure BrdU incorporation in the cecum and colorectum because no significant differences were observed in either the length or weight of these sites. Body weight gain and small intestine, cecum, and colorectum weights tended to be low in aGLP-2-treated rats; however, no statistically significant difference was

Table 1 *Growth and organ parameters of growing rats administered aGLP-2 or normal rabbit serum (NRS)*

	NRS	aGLP-2
Body weight gain (g)	117.7 ± 3.5	109.9 ± 7.4
Organ weight (g)		
Small intestine	6.87 ± 0.23	6.37 ± 0.40
Cecum	1.00 ± 0.03	0.92 ± 0.07
Colorectum	1.36 ± 0.06	1.14 ± 0.11
Organ length (cm)		
Small intestine	97.1 ± 1.9	91.2 ± 1.7*
Colorectum	16.4 ± 0.5	15.5 ± 0.5

Values are mean ± SEM (n = 6).
*Significantly different from the values of the NRS-treated group ($P < 0.05$).
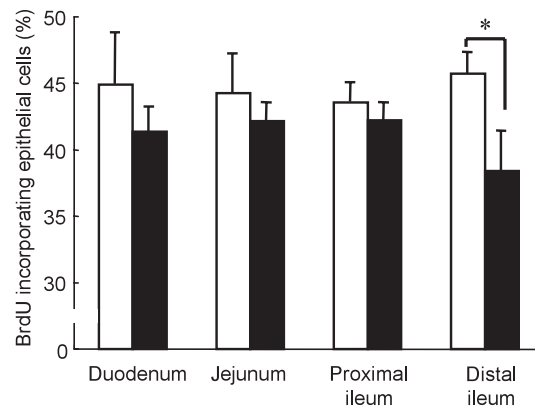

Fig. 1 BrdU incorporation of epithelial cells in the small intestine of weanling rats administered either aGLP-2 (solid bars) or normal rabbit serum (open bars) for 13 days. Values are mean and SEM. *Statistically significant difference between groups ($P < 0.05$, n = 6).

detected (Table 1).

Several reports using mature, adult animals have verified the intestinotropic effects of GLP-2 (1, 4). In this experiment, we sought to clarify the intrinsic effects of GLP-2 on the development of the intestinal epithelia in weanling rats. We found that administration of aGLP-2 suppressed BrdU incorporation in the epithelial cells and inhibited elongation of the small intestine in weanling rats. Exposure of L-cells to nutrient-rich chyme is important for intestinal growth (13). The growth rate of intestinal tissue is much higher during the suckling period than the post-weaning period (11). Previously, we found that GLP-2 was involved in intestinal growth during the suckling period in response to milk protein (7). The results of the present study also support the hypothesis that intrinsic GLP-2 contributes to intestinal growth during the weaning period, just after the

suckling period. During growth in the suckling and weaning period, the gastrointestinal tract, especially the small intestine, develops extensively. In contrast, there is almost no need for intestinal growth in adults. Endogenous GLP-2 plays a substantial role in intestinal development during both the suckling and weanling periods.

GLP-2 is thought to act in an endocrine manner. However, DPP-IV inactivates GLP-2 soon after release, and the clearance of GLP-2 is quite rapid (3). The plasma half-life of GLP-2 in the active form is estimated at approximately 7 min. Studies in pigs have shown that the metabolite of GLP-2 (3–33) is eliminated with a half-life of 22 min (6). In this study, inhibitory effects of aGLP-2 were observed at the distal ileum, where L-cells are densely localized (5). GLP-2 might remain at a relatively higher concentration in the distal small intestine than the proximal intestine because of the rapid inactivation of GLP-2. Our results suggest that endogenous GLP-2 preferentially acts in the distal section of the intestine in a paracrine manner during the weanling period. Thus, the effects of intrinsic GLP-2 on intestinal growth are probably limited to the distal ileum.

In conclusion, intrinsic GLP-2 promotes epithelial proliferation in the distal small intestine during the weanling period, thus indicating its contribution to the growth of the small intestine.

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