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Response of plasma glucagon-like peptide-2 to feeding pattern and intraruminal administration of volatile fatty acids in sheep

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ABSTRACT

Glucagon-like peptide-2 (GLP-2), a gut peptide secreted by enteroendocrine L cells, has recently been identified as a key regulator of intestinal growth and absorptive function in ruminants. However, reports on GLP-2 secretion are few, and more information regarding its secretion dynamics is needed. In this study, two experiments were conducted to elucidate the daily rhythm of GLP-2 secretion in response to feeding regimen and to investigate the effect of volatile fatty acids (VFA) on GLP-2 release in sheep. In experiment 1, blood samples were collected over 3 d from 4 Suffolk mature wethers adapted to a maintenance diet fed once daily; day 1 sampling was preceded by 24 h of fasting to reach steady state. On days 1 and 3, samples were collected every 10 min from 11:00 to 14:00 on both days and then every 1 h until 00:00 on day 1 only; feed was offered at 12:00. On day 2, feed was withheld, and sampling was performed every hour from 01:00 to 00:00. In experiment 2, 5 Suffolk mature wethers were assigned to 5 treatment groups of intraruminal administration of saline, acetate, propionate, butyrate, or VFA mix (acetate, propionate, and butyrate in a ratio of 65:20:15) in a 5 × 5 Latin square design. Blood samples were collected at 0, 1.5, 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 90, and 120 min relative to the beginning of administration at 12:00. In both experiments, plasma GLP-2, glucagon-like peptide-1 (GLP-1), glucose, insulin, and β-hydroxy butyric acid (BHBA) levels were measured. In experiment 1, incremental area under the curve was greater ($P < 0.05$) post-feeding than pre-feeding on days 1 and 3 for GLP-2 and tended to be greater ($P < 0.1$) on day 1 for GLP-1. Plasma insulin, glucose, and BHBA levels increased ($P < 0.05$) on day 1 post-feeding. Plasma GLP-2 was poorly correlated with GLP-1 but positively correlated with insulin, glucose, and BHBA. In experiment 2, administration of butyrate and VFA mix remarkably increased plasma GLP-2 ($P = 0.05$) and BHBA ($P < 0.0001$) levels compared with those in other treatments. Plasma GLP-1 levels were higher with butyrate administration compared with those in the saline, acetate, and VFA mix ($P = 0.019$). Propionate administration increased plasma glucose ($P = 0.013$) and insulin ($P = 0.053$) levels. Thus, our data confirmed that GLP-2 release is responsive to feeding and might be promoted by BHBA produced by the rumen epithelial metabolism of butyrate. Further molecular- and cellular-level studies are needed to determine the role of butyrate as a signaling molecule for GLP-2 release.

1. Introduction

Glucagon-like peptide-2 (GLP-2) is a gut peptide secreted by enteroendocrine L cells along with glucagon-like peptide-1 (GLP-1) [1] and plays an important role in

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the regulation of nutrient assimilation and energy homeostasis via the enhancement of gastrointestinal tract development, proliferation, and integrity [1,2]. In nonruminants, the role of GLP-2 is well documented as a specific gut growth factor [1–5]. Characterization of its precursor and receptor genes in bovine gastrointestinal tract [6,7] and reports of its intestinotrophic actions in calves [8–10] suggest that GLP-2 plays functional roles in ruminants as in nonruminants. Moreover, considerable attention is being paid to the application of GLP-2 as a management tool to improve gut development, function, and health in ruminants [11,12].

However, little is known about GLP-2–specific secretory stimuli in ruminants, although available information indicate that butyrate feeding may stimulate plasma GLP-2 release in calves [13,14]. Since GLP-2 is secreted along with GLP-1 from the same enteroendocrine L cells, and considering that they are both derived from the same gene [1], their secretory stimuli may be analogous. Ingested nutrients, especially carbohydrates (mainly glucose) and fat, have been reported as potent stimuli for intestinal secretion of GLP-1 and GLP-2 in nonruminants [15,16]. Volatile fatty acids (VFA), produced in the gut by bacterial fermentation of complex dietary carbohydrates, have been reported to increase intestinal proglucagon mRNA expression and secretion of glucagon-like peptides in nonruminants via the activation of specific G-protein–coupled receptors (GPCRs) expressed on enteroendocrine L cells [17,18]. Feeding/infusion of fats/VFA has been shown to increase the secretion of GLP-1 in ruminants [19–21]; however, whether such nutrients are involved in GLP-2 secretion is not yet known.

This study aimed to elucidate the daily rhythm of GLP-2 secretion in response to feeding regimen and to investigate the effect of intraruminal administration of VFA on GLP-2 release in sheep, in order to obtain basic knowledge on GLP-2 secretion pattern and its linkage to VFA production in ruminants.

2. Materials and methods

The procedures used in the present study were performed in accordance with the principles and guidelines for animal use established by Hiroshima University. All experimental procedures were approved by the Animal Care and Use Committee of Hiroshima University.

2.1. Animals, feeding pattern, treatment, and sample collection

2.1.1. Experiment 1

Four Suffolk mature wethers (41.3 ± 5.2 kg BW) were used to investigate the diurnal variation in plasma GLP-2 concentration in response to feeding regimen. Wethers were housed individually in metabolism crates and fed once daily at 12:00 a maintenance diet [22] consisting of alfalfa hay cubes and flaked barley (7:3) with free access to water and mineral blocks. After 2 wk of adaptation, sampling was conducted over three consecutive days, preceded by 24 h of fasting to ensure a steady-state condition. One day before sampling, a jugular vein catheter was fixed for sampling. During sampling days, sheep were fed on days 1 and 3, but feed was withheld on day 2. On day 1, sampling was initiated at 11:00, and feed was offered at 12:00, with samples collected every 10 min from 11:00 to 14:00 and then every 1 h until 00:00. On day 2, no feed was offered, and sampling was performed every 1 h starting from 01:00 until 00:00. On day 3, sampling was performed every 10 min from 11:00 until 14:00, with feed offered at 12:00. Therefore, days 1 and 3 were almost similar in fasting before sampling, but the sampling collection periods were different on both days. Blood samples (10 mL) were collected into tubes containing aprotinin (0.6 KIU/mL blood; Sigma-Aldrich Inc, Tokyo, Japan) and heparin (20 IU/mL blood; Wako Pure Chemical Industries Ltd, Osaka, Japan) and centrifuged at 2,340 × *g* for 15 min at 4°C; plasma samples were maintained at −80°C until assayed.

2.1.2. Experiment 2

Five Suffolk mature wethers (40.2 ± 4.5 kg BW) were used to elucidate the effect of VFA on GLP-2 secretion. Wethers housing and feeding management were similar to those in experiment 1. After 2 wk of adaptation, wethers were randomly assigned to 5 treatments of intraruminal administration in a 5 × 5 Latin square design as follows: saline (control), acetate, propionate, butyrate, and a mix of VFA at a ratio of 65:20:15 for acetate, propionate, and butyrate, respectively. The infused amounts of VFA, individually and mixed, were adjusted to provide 10% of maintenance energy requirements [22]. The VFA solutions were prepared by dissolving the respective amounts of sodium acetate, sodium propionate, and sodium butyrate (Wako Pure Chemical Industries Ltd, Osaka, Japan) into 150 mL of distilled water. Each solution was injected into the rumen, via a rumen cannula, over 1 min by using a 150 mL plastic syringe. Blood samples (10 mL) were collected at 0, 1.5, 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 90, and 120 min relative to the beginning of the injection (at 12:00 = feeding time) and were prepared as mentioned in experiment 1.

2.2. Plasma hormone and metabolite analyses

Plasma hormone concentrations were measured using the competitive time-resolved fluoroimmunoassay (TR-FIA) technique by using a 2030 Multilabel Reader, ARVO X4 (PerkinElmer, Inc, Waltham, MA, USA). Insulin was assayed as described by Takahashi et al [23] by using europium (Eu)-labeled synthetic bovine insulin and polystyrene microtiter strips (Nalge Nunc Int., Tokyo, Japan) coated with anti-guinea pig γ-globulin. Intra-assay and interassay coefficients of variation (CVs) averaged 2.2% and 1.8%, respectively. The least detectable dose (LDD) and 50% inhibitory concentration (IC_{50}) of insulin were 0.016 ng/mL and 1.073 ng/mL, respectively.

Bioactive GLP-1 was determined as described previously by Fukumori et al [21] by using Eu-labeled human GLP-1 and polystyrene microtiter strips coated with anti-rabbit γ-globulin. Intra-assay and interassay CVs averaged 1.7%