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Characterization of glucagon-like peptide 2 pathway member expression in bovine gastrointestinal tract

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ABSTRACT

Glucagon-like peptide 2 (GLP-2), secreted by enteroendocrine cells, has several physiological effects on the intestine of monogastric species, including promotion of growth of intestinal epithelium, reduction of epithelial cell apoptosis, and enhancement of intestinal blood flow, nutrient absorption, and epithelial barrier function. The regulatory functions of GLP-2 in the ruminant gastrointestinal tract (GIT) have not been well studied. The objectives of this investigation were to characterize the mRNA expression of 4 members of the GLP-2 pathway throughout the bovine GIT, including (1) proglucagon (*GCG*), the parent peptide from which GLP-2 is derived through cleavage by prohormone convertase; (2) prohormone convertase (*PCSK1*); (3) GLP-2 receptor (*GLP2R*); and (4) dipeptidyl peptidase IV (*DPP4*), the enzyme that inactivates GLP-2. Gene expression was evaluated in rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, and rectum collected at slaughter from prepubertal heifers, mature cows in early, mid, and late lactation, and nonlactating cows (n = 3 per stage) by a gene expression profiling assay. In addition, mRNA expression of 14 genes involved in nutrient transport, enzyme activity, blood flow, apoptosis, and proliferation were evaluated in the 9 GIT tissues for their association with *GCG* and *GLP2R* mRNA expression. Immunohistochemistry was used to localize GLP2R protein in tissues of the lower GIT. Results indicated that mRNA expression of *GCG*, *PCSK1*, *GLP2R*, and *DPP4* varies across the 9 GIT tissues, with greatest expression in small and large intestines, and generally nondetectable levels in forestomachs. Expression of *DPP4* and *GLP2R* mRNA varied by developmental stage or lactational state in intestinal tissues. Expression of *GCG* or *GLP2R* mRNA was correlated with molecular markers of proliferation, apoptosis, blood flow, enzyme activity, and urea transport, depending on the tissue examined, which suggests a potential for involvement of GLP-2 in these physiological processes in the ruminant GIT. The GLP2R protein was expressed in intestinal crypts of the bovine GIT, which is consistent with the distribution in monogastric species. Our findings support a functional role of the GLP-2 pathway in bovine GIT and the potential for use of GLP-2 as a therapy to improve intestinal function and nutrient absorption in ruminants.
Key words: cattle, gastrointestinal tract, gene expression, glucagon-like peptide 2

INTRODUCTION

Glucagon-like peptide 2 (**GLP-2**) is a hormone secreted by intestinal L cells, which has been shown in monogastric species to increase cell proliferation in the intestinal crypts, reduce apoptosis of intestinal mucosa, suppress inflammation, and enhance nutrient absorption in the gut (Lovshin and Drucker, 2000; Burrin et al., 2003; Drucker, 2005). The peptide has been used to improve intestinal growth and integrity after injury or after regression resulting from parenteral nutrition in pigs, and may be a viable treatment to improve intestinal function in premature infants (Burrin et al., 2007), as well as adults with short-bowel syndrome (Jeppesen, 2003).

The biological actions of GLP-2 are determined by the liberation of the peptide from its parent protein proglucagon (**GCG**) via the enzyme prohormone convertase (**PCSK1**), its degradation by dipeptidyl peptidase IV (**DPP4**), and interaction with its G protein-coupled receptor, GLP-2 receptor (**GLP2R**). To date, functional studies of the GLP-2 pathway in livestock have been limited to swine, although preliminary reports indicate that *GCG* and *GLP2R* mRNA are expressed in bovine gastrointestinal tract (**GIT**; Taylor-Edwards et al., 2008), and that GLP-2 treatment enhances splanchnic blood flow and mass of the small intestine in calves (Taylor-Edwards et al., 2009a,b). The complete functions and therapeutic potential of GLP-2 in ruminants are unknown, but its use as a treatment for intestinal diseases such as calf scours has been suggested (Burrin et al., 2003).

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The purpose of this study was to evaluate mRNA expression of *GCG*, *PCSK1*, *DPP4*, and *GLP2R*, as well as mRNA for nutrient transporters, markers of apoptosis, proliferation, growth, enzyme activity, and blood flow in the bovine GIT. Gene expression was evaluated during 5 stages of development or lactation, including prepubertal heifers, lactating cows during early, mid, and late lactation, and nonlactating cows. A 22-gene multiplex expression assay was used to test the hypothesis that changes in lactational stage are associated with changes in expression of GLP-2 pathway members. In addition, we tested the hypothesis that expression of *GCG* or *GLP2R* mRNA is correlated to changes in expression of mRNA for markers of apoptosis, proliferation, growth, enzyme activity, and blood flow, because GLP-2 is known to affect these processes in other species. Last, cellular expression of GLP2R protein was characterized in the lower bovine gut by immunohistochemistry.

MATERIALS AND METHODS

Bovine Tissues

Intestinal tissues including rumen, reticulum, omasum, abomasum, duodenum, jejunum, ileum, cecum, and rectum were collected at slaughter from culled adult Holstein cows from the Beltsville Agricultural Research Center dairy herd (Beltsville, MD) at the institutional abattoir, and from prepubertal Holstein heifers (100 to 130 d of age) from a study conducted at Clemson University (Clemson, SC). All animal procedures associated with the heifer samples were approved by the Clemson University Animal Care and Use Committee. For each developmental stage examined, gene expression was based on 3 animals. Among the lactating Holsteins, cows were categorized as early lactation [19 to 92 DIM; 3.7 ± 1.4 yr of age (mean ± SD); lactation number of 2.3 ± 1.5 (mean ± SD)], mid lactation (138 to 201 DIM; 4.9 ± 4.1 yr of age; 3.0 ± 3.5 lactations), or late lactation (250 to 414 DIM; 4.9 ± 2.1 yr of age; 2.7 ± 2.1 lactations). Nonlactating cows were 5.3 ± 2.1 yr of age, nonpregnant, and killed either 59 or 77 d after cessation of milking.

At slaughter, each tissue was dissected and rinsed in tap water (rumen, reticulum, omasum, and abomasum) or physiological saline (duodenum, jejunum, ileum, cecum, and rectum) to remove residual feed and GIT contents. In an attempt to maintain consistency for tissue sampling, rumen was sampled from the anterior portion beneath the reticulum and below the rumen fluid layer; abomasum was sampled from the lower, ventral surface of the posterior compartment; duodenum was sampled posterior to the bile ducts; ileum was sampled anterior to the ileo-cecal junction; cecum was sampled from the most distal region of the enclosed sack; and rectum was sampled anterior to the anus.

For RNA analysis, sampling was limited to the epithelial layers. For the small and large intestinal segments, a glass slide was used to scrape the epithelial mucosal layer for sample collection. Samples were then immediately immersed in RNAlater RNA stabilization solution (Ambion, Austin, TX) and stored at 4°C overnight. Excess RNAlater was then removed and preserved tissues were stored at −80°C until RNA extraction. Whole tissues were also sampled for sectioning and subsequent immunohistochemical analysis. Tissue pieces were placed in glass scintillation vials with 15 mL of 10% neutral buffered formalin (pH 6.8 to 7.2; Richard-Allan Scientific, Kalamazoo, MI) and stored at 4°C overnight. Excess neutral buffered formalin was removed and tissue pieces were rinsed once with 70% ethanol, and then stored at 4°C in fresh 70% ethanol until paraffin embedding and tissue sectioning.

RNA Extraction

Frozen tissues were homogenized in Trizol reagent (Invitrogen, Carlsbad, CA), and total RNA was extracted using the RNeasy Midi Kit (Qiagen, Valencia, CA) with on-column DNase digestion. Quality of RNA was assessed using a 2100 Bioanalyzer and RNA 6000 Nano LabChip kits (Agilent Technologies, Palo Alto, CA), and RNA concentration was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE).

22-Gene Multiplex Expression Assay

Expression of 22 genes was quantified simultaneously in each RNA sample using a custom multiplex gene expression assay, based on traditional reverse transcription-PCR, developed for the Genome Lab GeXP Genetic Analysis System and GeXP Start Kit (Beckman Coulter, Inc., Brea, CA). Genes of interest are summarized in Table 1, including 4 potential reference genes for normalization: ATP synthase (***ATP5B***), hypoxanthine guanine phosphoribosyl transferase 1 (***HPRT1***), *GAPDH*, and β-2 microglobulin (***B2M***). Primer selection was conducted using eXpress Designer software (Beckman Coulter, Inc.) and GenBank accession numbers (http://www.ncbi.nlm.nih.gov/) of bovine transcripts of interest. In each case, we confirmed that the primers used for each gene produced a single product of the expected size as measured by capillary electrophoresis. Primers were evaluated in both singlet and multiplex reactions to verify that the same amplicons appeared (at the same sizes) in the electropherogram in the multiplex reaction as they appeared in the singlet

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