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The developmental plasticity of colocalization pattern of peptide YY and glucagon-like peptide-1 in the endocrine cells of bovine rectum

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ABSTRACT

Peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) are produced in endocrine cells that show distal distribution in each of small and large intestine. They are colocalized in the same endocrine cells at different ratios depending on the animal species. The present study examined the possibility of plasticity in the colocalization pattern in the bovine rectum, which is known to contain endocrine cells at a high concentration. Consecutive sections from different pre- and postnatal stages were stained immunohistochemically. The immunoreactive (IR) cells were divided into three groups: 1) cells IR for both PYY and GLP-1 (PYY/GLP-1-IR cells), 2) cells IR only for PYY (PYY-IR cells), and 3) cells IR only for GLP-1 (GLP-1-IR cells). The percentage of PYY/GLP-1-IR cells was high in the prenatal (early, mid- and late fetuses) and suckling stages, whereas it decreased in the herbivorous (weaning, weaned and adult) stages. In contrast, percentages of PYY- and GLP-1-IR single cells were low in the prenatal and suckling stages and increased after the suckling stage. PYY/GLP-1 endocrine cells may adapt to the change of digestion depending on feeding habits and/or specific developmental stages of cattle. The present results suggest the developmental plasticity of the colocalization pattern of gut hormones with nutritional transition.

Representative gut hormones that are assumed to have roles in feeding control are peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) (2, 12, 13). PYY is a 36 amino acid straight chain polypeptide belonging to the pancreatic polypeptide family (2, 8). GLP-1 is a peptide hormone belonging to glucagon super-family and produced from the same precursor as glucagon and glicentin (4, 5). PYY and GLP-1 are synthesized and released from a special type of entero-endocrine cells (2, 12). These cells are also referred as the L type of endocrine cells because of the large size of their secretory granules in most animal species (2, 9, 11). A tendency for an increase of L cells along the gut is reported in the large intestine, with the highest density in the rectum (5). Endocrine cells including glucagon-like immunoreactive (IR) cells and PYY-IR cells are abundant in the large intestine, particularly in the rectum (6, 11).

Colocalization of PYY and proglucagon-derived peptides (glicentin and GLP-1) in gut endocrine cells has been reported at different ratios in various animal species (1–3, 5, 9, 11). However, detailed study on the colocalization of PYY and GLP-1 in the different ontogenetic stages and the plasticity of the colocalization has not been conducted yet. Therefore, evidence is needed to clarify the plasticity of their colocalization depending on the developmental stages of animals. Ruminants have two drastic changes in nutritional stages: suckling and herbivore, which occurs from fetal to postnatal stages, and from suckling to herbivorous, respectively. The pres-

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ent study was aimed to investigate the colocalization patterns and developmental plasticity of PYY and GLP-1 in the bovine rectum at different ontogenetic stages using immunohistochemical methods.

MATERIALS AND METHODS

Subjects. Twenty-one Holstein cattle at seven different developmental stages, early fetus: 20–40 cm in crown-rump-length (CRL, n = 3), mid-fetus: 41–70 cm (n = 3), late fetus: 71–100 cm (n=3), suckling calf (1- to 2-week-old, n = 3), weaning calf (2-month-old, n = 3), weaned calf (10-month-old, n = 3) and adult (1- to 8-year-old, n = 3) were used in this study. Postnatal animals were exsanguinated from the carotid artery under anesthesia (xylazine hydrochloride 0.3 mg/kg and thiopental 7 mg/kg). Prenatal animals were obtained at the autopsy of their anesthetized mother for pathological inspection. The present experiment was carried out in line with the guidelines of the Committee of Animal Experiments of Obihiro University of Agriculture and Veterinary Medicine (No. 17–51, 20–95, 21–108, 23–46).

Immunohistochemistry. Tissue samples from the rectum just proximal to the anorectal line were taken after dissection and fixed in Bouin's solution for 24 h. The samples were processed conventionally for paraffin sections, which were cut serially at 2 μm and mounted on gelatin-coated slides. For immunohistochemical staining, the sections were deparaffinized and rinsed in water. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol. Then, the samples were washed three times with phosphate-buffered saline (PBS, pH 7.4), and incubated in normal goat serum (1 : 50, S-1000; Vector Laboratories Inc., Burlingame CA, USA) for 30 min at room temperature (RT). The sections were again washed with PBS and incubated with one of the primary antisera raised in rabbits against porcine PYY (1 : 10,000, IHC7173; Peninsula Lab. Inc., Belmont, USA) and human GLP-1 (1 : 10,000, Y-320; Yanaihara Institute, Shizuoka, Japan) and incubated at 4°C overnight. On the second day, the sections were washed with PBS and incubated with biotinylated anti-rabbit IgG in goat (1 : 200, BA-1000; Vector Laboratories Inc.) at RT for 30 min. After washing again with PBS the sections were further incubated with avidin-biotin-peroxidase complex (1 : 2, PK-6100, Vectastain *Elite* ABC kit; Vector Laboratories). Reacted sites were visualized with Tris buffer (pH 7.4) containing 0.02% 3, 3'-diaminobenzidine tetrahydrochloride (Dojindo Inc., Kumamoto, Japan) and 0.03% H_2O_2. Then, the sections were lightly counterstained with Mayer's hematoxylin, and mounted through conventional steps. Negative control for the immunohistochemistry was carried out by incubating sections with non-immune rabbit serum or PBS instead of the primary antiserum. Specific immunoreactions were not detected in these sections.

PYY- and GLP-1-IR cells in the consecutive sections were observed and photographed under a light microscope with differential interference optics (Nikon, Tokyo, Japan) and microphotographs were taken with a digital camera (DS-5M, Nikon). The percentage of colocalized (PYY/GLP-1) and non-colocalized (single PYY and GLP-1) cells were evaluated at all developmental stages. In total, 530 immunoreactive cells from 21 animals of seven stages were counted and the percentages of PYY/GLP-1-, PYY- and GLP-1-IR cells in each stage were calculated.

Statistical analysis. The obtained data are expressed as the mean ± SEM. The statistical analysis of the difference between mean values was performed by one-way ANOVA and Tukey's multiple comparison tests using GraphPad Prism (Version 5.00 for Windows; GraphPad Software, San Diego California USA). Statistical significance was assigned at $P < 0.05$.

RESULTS

PYY- and GLP-1-IR cells were detected abundantly in the crypt, being more abundant at the base of the crypt. They were more numerous in younger animals. The present study focuses on their colocalization pattern. Three types of IR cells were observed in the present study (Fig. 1): *1)* cells showing immunoreactivities for both PYY and GLP-1 are expressed simply as PYY/GLP-1-IR cells, *2)* cells showing immunoreactivity only for PYY and not for GLP-1 are expressed as PYY-IR cells, and *3)* cells showing immunoreactivity only for GLP-1 and not for PYY are expressed as GLP-1-IR cells. These three types of endocrine cells were observed in all developmental stages at different percentages (Fig. 2). The most remarkable differences were observed in PYY/GLP-1-IR cells among the different developmental stages. The percentage of PYY/GLP-1-IR cells was significantly higher in the fetal (early, mid- and late) and suckling stages (64–76%, average 72%) and decreased drastically in the weaning, weaned and adult stages (17–34%, average 26%). No significant differences of PYY/GLP-1-IR cells were found from the early fetal to the suckling stages and from the weaning to adult stages. The per-

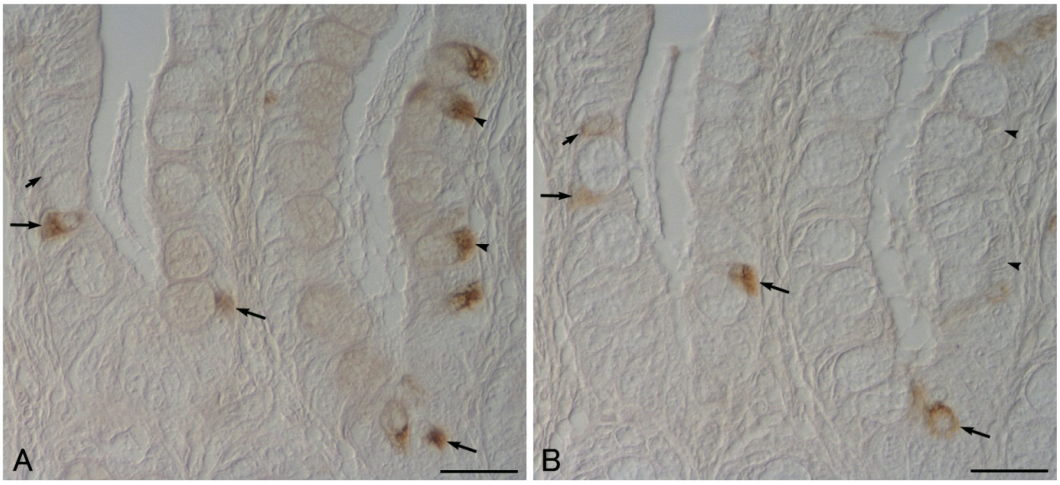

Fig. 1 PYY/GLP-1-immunoreactive cells (large arrows), PYY-immunoreactive cells (arrowheads), and GLP-1-immunoreactive cells (small arrows) in consecutive sections immunostained for PYY (**A**) and GLP-1 (**B**). Rectum of cattle fetus (crown-rump-length 66 cm). Bars: 20 µm

centage of GLP-1-IR cells was low in the fetal and suckling stages (7–29%, average 18%) and increased in the herbivorous stages (weaning, weaned, adult; 49–58%, average 54%). The percentage of PYY-IR cells was higher in the period after suckling (17–25%, average 20%) in contrast to that in the period before weaning (6–19%, average 10%).

DISCUSSION

Colocalization of PYY and GLP-1 was proven in the endocrine cells of bovine rectum. Previous immunohistochemical studies on rodents and humans have reported the colocalizaiton of PYY with enteroglucagon or proglucagon-derived peptides such as glicentin and GLP-1 (1–4, 9). In the present study, the percentages of colocalization changed with the development of cattle. It has been reported that cells expressing both PYY and glucagon appeared in the endocrine pancreas of rat at the transition stages (late fetus and early postnatal) of development (7). Such multihormonal cells were not detected in the mature pancreas. On the other hand, cells expressing both PYY and proglucagon-derived peptides were reported as typical subpopulations of enteroendocrine cells in the distal large intestine of mouse, rat, pig and human (1, 3, 10). It is tempting to associate the pathway variations of cellular differentiation of PYY/GLP-1 cells with the ontogenetic differentiation depending on the regional difference of gut.

However, no study has reported different patterns of PYY and GLP-1 colocalization of gut endocrine cells in different developmental stages. In the present study, PYY and GLP-1 immunoreactivities were abundantly colocalized in different bovine developmental stages. The PYY/GLP-1 colocalization was observed in over 70% of counted cells in the prenatal and suckling stages and had no significant differences throughout those stages. However, the percentage of PYY/GLP-1 endocrine cells was significantly decreased in the herbivorous (weaning, weaned and adult) stages. An interesting finding in this study is the high percentage of PYY/GLP-1 endocrine cells in the suckling stage in addition to the prenatal stage. It is possible that the regulatory mechanism of the gastrointestinal tract in the suckling stage is different from that of animals at the herbivorous stages. In ruminants, marked morphological and functional changes occur around the weaning transition. The percentages of PYY- and GLP-1-IR single endocrine cells were also different among developmental stages. GLP-1-IR single cells increased drastically after the suckling stage, whereas the changes of PYY-IR cells were not as conspicuous as those of PYY/GLP-1- and GLP-1-IR cells. The change and decrease of PYY/GLP-1 (colocalized)-IR cells at the weaning could be related to the adaptation of the regulatory mechanism for the herbivorous nature of the digestion. Functional differentiation may occur from PYY/GLP-1 cell to PYY and/or GLP-1 cells as developmental physiology. It is possible that the differentiation occurs actively at the weaning stage.

The present study revealed the developmental plasticity of colocalization pattern of PYY and GLP-1 in endocrine cells of the bovine rectum. PYY/GLP-1-IR cells might play different physiological roles

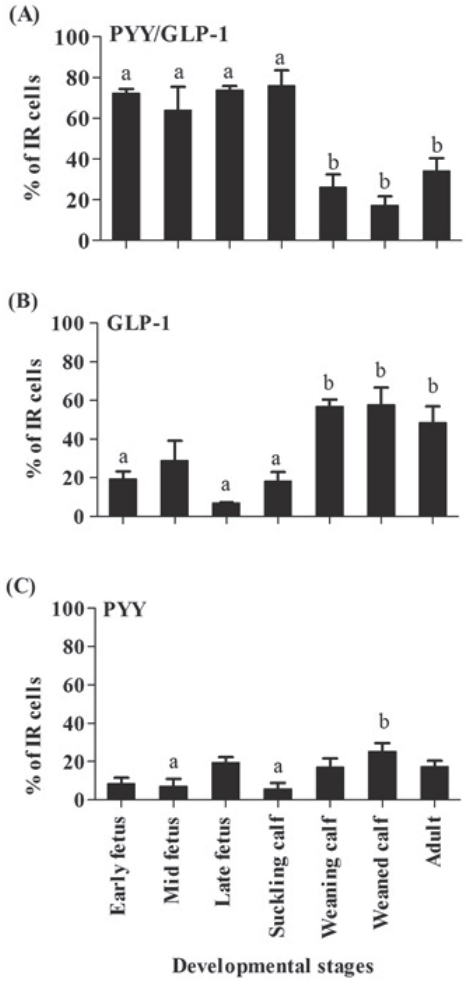

Fig. 2 Percentages of PYY- and GLP-1-immunoreactive endocrine cells in the bovine rectum at different developmental stages. **A**: PYY/GLP-1 (colocalized) cells, **B**: GLP-1 single cells, **C**: PYY single cells. The data represent mean ± SEM. Significant differences were observed between results labeled with different characters, **a** and **b** ($P < 0.05$).

depending on feeding habits and specific developmental stages of animals. In this context, regulatory roles of these peptides in the feeding mechanism of ruminant are highly noteworthy.

REFERENCES

1. Ali-Rachedi A, Varndell IM, Adrian TE, Gapp DA, Van Noorden S, Bloom SR and Polak JM (1984) Peptide YY (PYY) immunoreactivity is co-stored with glucagon-related immunoreactants in endocrine cells of the gut and pancreas. *Histochemistry* **80**, 487–491.
2. Ballantyne GH (2006) Peptide YY (1–36) and peptide YY (3–36): Part 1. Distribution, release and action. *Obes Surg* **16**, 651–658.
3. Böttcher G, Sjölund K, Ekblad E, Håkanson R, Schwartz TW and Sundler F (1984) Coexistence of peptide YY and glicentin immunoreactivity in endocrine cells of the gut. *Regul Pept* **8**, 261–266.
4. Holst JJ and Orskov C (1994) Glucagon and other proglucagon-derived peptides, In: *Gut Peptides: Biochemistry and Physiology.* (Walsh JH and Graham JD, eds), pp305–340, Raven Press, New York.
5. Kieffer TJ and Habener JF (1999) The glucagon-like peptide. *Endocr Rev* **20**, 876–913.
6. Kitamura N, Yamada J, Calingasan NY and Yamashita T (1985) Histologic and immunocytochemical study of endocrine cells in the gastrointestinal tract of the cow and calf. *Am J Vet Res* **46**, 1381–1386.
7. Larsson L-I (1998) On the development of the islets of Langerhans. *Microsc Res Tech* **43**, 284–291.
8. Mannon P and Taylor IL (1994) The pancreatic polypeptide family, In: *Gut Peptides: Biochemistry and Physiology*. (Walsh JH and Graham JD, eds), pp341–370, Raven Press, New York.
9. Nilsson O, Bilchik AJ, Goldenring JR, Ballantyne GH, Adrian TE and Modlin MI (1991) Distribution and immunocytochemical colocalization of peptide YY and enteroglucagon in endocrine cells of the rabbit colon. *Endocrinology* **129**, 139–146.
10. Roth KA, Kim S and Gordon JI (1992) Immunocytochemical studies suggest two pathways for enteroendocrine cell differentiation in the colon. *Am J Physiol* **263**, G174-G180.
11. Sundler F and Håkanson R (1988) Peptide hormone-producing endocrine/paracrine cells in the gastro-entero-pancreatic region, In: *Handbook of Chemical Neuroanatomy. Vol. 6: The Peripheral Nervous System.* (Björklund D, Hökfelt T and Owman C, eds), pp219–295, Elsevier Science Publishers B. V., Amsterdam.
12. Stanley S, Wynne K and Bloom S (2004) Gastrointestinal satiety signals III. Glucagon-like peptide 1, oxyntomodulin, peptide YY, and pancreatic polypeptide. *Am J Physiol Gastrointest Liver Physiol* **286**, 693–697.
13. Waren AM and Bloom SR (2007) Gut hormones and appetite control. *Gastroenterology* **132**, 2116–2130.