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An Immunohistochemical Study of Endocrine Cells in the Abomasum of Vagotomized Calf

Raden Harry SOEHARTONO[1)]*, Nobuo KITAMURA[2)]**, Norio YAMAGISHI[1)], Kiyoshi TAGUCHI[3)], Junzo YAMADA[2)] and Haruo YAMADA[1)]

[1)]Departments of Veterinary Surgery and [2)]Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080–8555 and [3)]Department of Veterinary Surgery II, Faculty of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069–8501, Japan

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ABSTRACT. The effect of thoraco-vagotomy on the distribution and frequency of chromogranin-, serotonin-, somatostatin- and gastrin-immunoreactive cells in the abomasum of the calf were investigated by immunohistochemistry. Calves were vagotomized at 1 week old and sampled 2 and 4 weeks later. The endocrine cells generally decreased in number in vagotomized calves as compared to non-operated control calves. However, the detailed responses of endocrine cells to vagotomy varied depending on the endocrine cell type, region of gastric mucosa, and period after vagotomy. The present result suggests that the vagus nerve has an influence on the intrinsic regulatory system by endocrine cell control in the ruminant abomasum.

KEY WORDS: abomasum, calf, endocrine cell, immunohistochemistry, vagotomy.

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The abomasum is the fourth compartment of the ruminant stomach and the glandular portion which is equivalent to that of monogastric animals. The abomasum in the milk-fed calf as a preruminant animal is more important than the still undeveloped forestomach.

The parasympathetic innervation by the vagus nerve constitutes an important link between the central nervous system and the gastrointestinal tract [21], and it has a significant influence on the secretory function of the stomach as well as on motility [3, 8, 10, 13, 19, 20]. The vagus nerve has a relatively much greater importance in the control of gastric activity in ruminants than in other species [18], and its malfunction might cause serious disturbances in the activity of both the abomasum and the forestomach (Hoflund's syndrome).

Vagotomy has an effect not only on the motility of the stomach [3, 8, 10, 24], but also on secretion [13, 19, 20] and intramural nervous elements [24]. This effect also might influence the activity of endocrine cells, which are an important element of the intrinsic regulatory systems in the digestive tract. The number of endocrine cells after vagotomy has been described in several monogastric animals [15, 19, 22]. Contradictory results have been reported about the change in the number of endocrine cells such as chromogranin, serotonin, gastrin and somatostatin cells, after vagotomy [22, 23, 26]. These reports suggested that vagotomy has different effects depending on the animal species and the types of endocrine cells. An investigation of the effect of vagotomy on ruminant gastric endocrine cells would be interesting because this species has a different dependency of gastric activity on the vagus from the monogastric animals [18].

* PRESENT ADDRESS: SOEHARTONO, R. H., Department of Veterinary Clinics, Bogor Agricultural University, Bogor 16710, Indonesia.
**CORRESPONDENCE TO: KITAMURA, N., Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080–8555, Japan.

The present study investigated the possible changes in endocrine cells due to vagotomy in the abomasum of the calf.

MATERIALS AND METHODS

Animals: A total of 17 dairy calves were divided into 2 groups: control (3 calves each at 1, 3 and 5 weeks of age), and vagotomized (4 calves each at 3 and 5 weeks of age and at 2 and 4 weeks after vagotomy, respectively). The animals were bottle-fed with 2 *l* milk (10% w/v commercial milk replacement food) twice a day from 1 week of age. Roughage was withheld.

Vagotomy: Under general anesthesia, a total thoraco-vagotomy was undertaken at 1 week old through a left-side thoracic incision at the level of the 7th rib. Both sides of the dorsal and ventral vagi were cut 3 cm in length at the level of the 7th rib. The results of vagotomy were confirmed at necropsy.

Preparation of abomasal samples: After slaughter, a tissue sample was collected from each of the 4 regions, i.e., the corpus and pyloric regions of the lesser and greater curvatures of the abomasum. They were fixed in phosphate buffer (pH 7.4) containing 10% formalin, and processed routinely for embedding in paraffin.

Immunohistochemical technique: Sections were cut 5 μm in thickness, mounted on poly-L-lysin coated slides and processed for immunohistochemistry. The primary antisera used are anti-chromogranin (1:10,000; Lot. 410202, INCSTAR., Stillwater, MN, U.S.A.), anti-serotonin (1:10,000; Sero-2–3, Dr. J. Nishitsutsuji-Uwo, Shionogi Co., Kyoto, Japan), anti-somatostatin (1:9,000; Lot.27092, Immuno Nuclear Corporation, Stillwater, MN, U.S.A.) and anti-gastrin (1:5,000; GP1304, Dr. N. Yanaihara, Yanaihara

Institute, Shizuoka, Japan). The site of antigen-antibody reaction was visualized by the avidin-biotin-peroxidase complex method [14] using commercially available kit (Vectastain *Elite* ABC kit, PK-6100, Vector Laboratories Burlingame, U.S.A.) and were developed with 3,3'-diaminobenzidin tetrahydrochloride solution containing 0.03% H_2O_2 followed by slight counter staining with Mayer's haematoxylin.

The antisera were diluted in 0.01 M phosphate buffered 0.1 M saline (pH 7.3), which was recommended to prevent non-specific binding of immunoglobulins by ionic interactions [9]. Negative control was carried out by incubating sections with the diluent and normal serum instead of the primary antiserum (see Fig. 2B) and positive control was conducted with sections of the ruminant which is previously confirmed [1, 2, 4, 17].

Quantification: Immunoreactive endocrine cells of the mucosa were displayed in the TV monitor through CCD camera attached to the microscope equipped with $\times$ 20 objective lens. The number of endocrine cells was counted and expressed as the mean $\pm$ SEM per unit area (13.05 μm^2), and differences between corresponding region were analyzed using Mann-Whitney test. $P<0.05$ was considered statistically significant.

RESULTS

The endocrine cells were distributed throughout the glands and the superficial epithelium. They were mostly distributed in the basal portions of the glands in the corpus regions, and were found to spread over the glands in the pyloric region. The relative frequencies of immunoreactive endocrine cells are summarized in Fig. 1.

Corpus region: In the control animals, chromogranin-immunoreactive (IR) cells in the lesser curvature increased in number steadily with age (P=0.0457 between 1 and 5 weeks old). On the other hand, they decreased in the greater curvature (P=0.0498 between 1 and 5 weeks old).

The number of chromogranin-IR cells in the lesser curvature of the corpus region decreased significantly (P=0.0373 compared to control of the same age) 2 weeks after vagotomy (3 weeks old) (Fig. 2), then further decreased (P=0.0028 compared to control) 4 weeks after vagotomy (5 weeks old). This pattern is not so conspicuous in the greater curvature at either 3 or 5 weeks old. The frequency of serotonin- and somatostatin-IR cells in the corpus region was not significantly influenced by the vagotomy.

Pyloric region: In control animals, chromogranin- and gastrin-IR cells showed changes in frequency with age. Chromogranin-IR cells in the lesser curvature increased steadily with age (P=0.0210 between 1 and 5 weeks old and P=0.0244 between 3 and 5 weeks old), while the number did not change significantly in the greater curvature. Gastrin-IR cells in the lesser curvature decreased significantly (P=0.0504) in number from 1 to 5 weeks old.

In the vagotomized calves, the number of chromogranin-IR cells in the lesser curvature decreased significantly 2 weeks (P=0.0089) and highly significant 4 weeks (P=0.0020) after vagotomy compared to control. They decreased significantly (P=0.0317 compared to control) at 5 weeks old in the greater curvature. The number of serotonin-IR cells in the lesser curvature decreased highly significant at 3 and 5 weeks old (P=0.0033 and 0.0004, respectively, compared to control). In the greater curvature there was no significant difference at 3 and 5 weeks old. Somatostatin-IR cells showed results similar to serotonin-IR cells, i.e., in the lesser curvature they decreased significantly at 3 and 5 weeks old (P=0.0223 and 0.0528, respectively, compared to control). Gastrin-IR cells decreased significantly in the lesser (P=0.0084 compared to control) and greater (P=0.0414 compared to control) curvatures at 3 weeks old (Fig. 3), whereas there was no significant difference at 5 weeks old in either curvature.

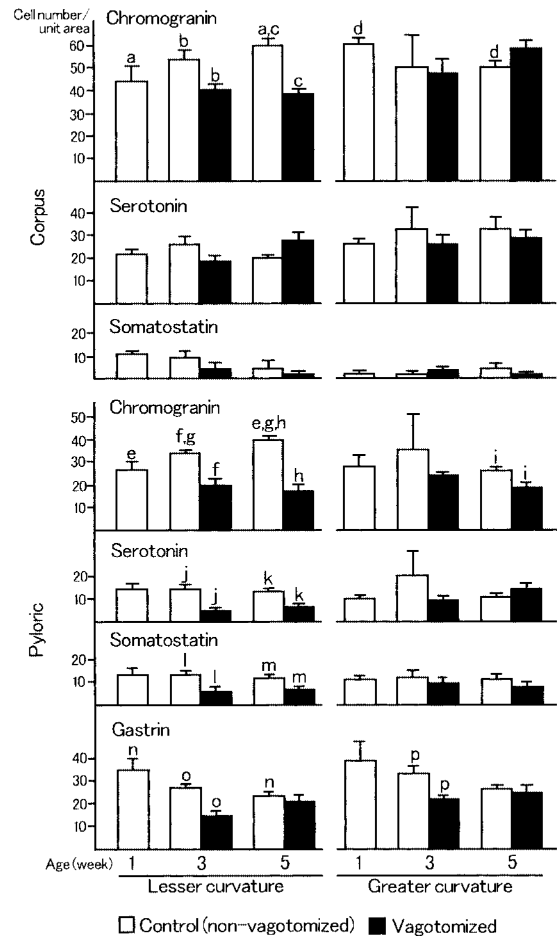

Fig. 1. The frequency of endocrine cells per unit area (13.05 μm^2) of mucosa in the corpus and pyloric region of abomasum of control (non-vagotomized) and vagotomized calves. Each bar represents the mean+SEM. Significant differences were detected between the same alphabet on the bar ($P<0.05$: a, b, d, e, g, i, l, m, n, $P<0.01$: c, f, h, j, k, o, p).

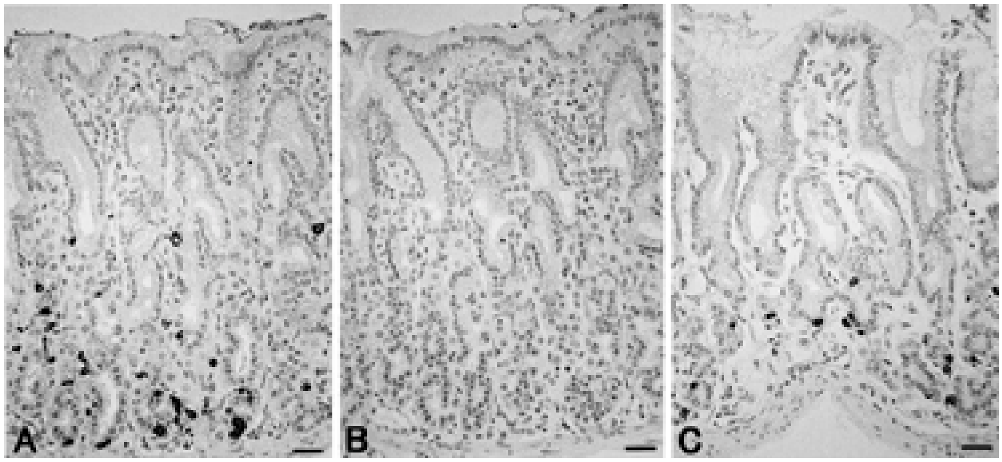

Fig. 2. Chromogranin-immunoreactive cells in the corpus region of lesser curvature of non-vagotomized (A) and vagotomized calves (C) at 3 weeks old. Replacement of the specific antiserum with normal rabbit serum shows no staining in the serial section to A (B). Scale bars=30 μm.

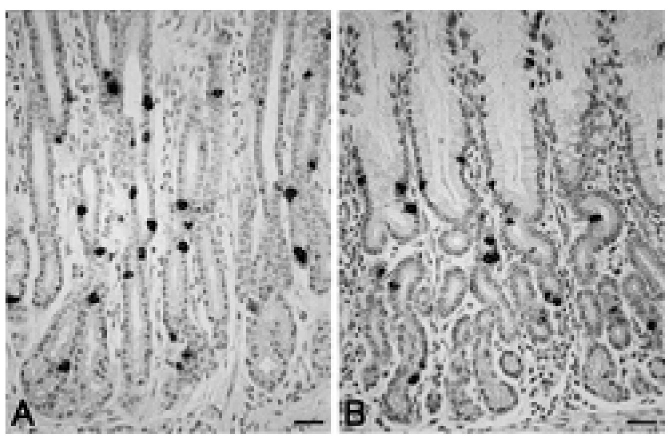

Fig. 3. Gastrin-immunoreactive cells in the pyloric region of greater curvature of non-vagotomized (A) and vagotomized calves (B) at 3 weeks old. Scale bars=30 μm.

DISCUSSION

This is the first report on the effect of vagotomy on the endocrine cells in the ruminant stomach. The distribution and frequency of occurrence of chromogranin-, serotonin-, somatostatin- and gastrin-IR endocrine cells were clarified in the abomasum of normal and vagotomized calves. These endocrine cell types are typical of the ruminant glandular stomach, the abomasum [1, 2, 4, 17]. Chromogranin immunoreactivity could be a general marker of gut endocrine cells [7, 16].

In normal calves investigated during the present study period (1–5 weeks), the frequency of endocrine cells was stable or slightly decreased with age in accordance with a previous report [17]. These tendencies in the restricted unit area may be attributable to the growth of the gastric wall particularly for the greater curvature. However, it can not be explained by this attribution that chromogranin-IR cells increased in the lesser curvature in both the corpus and pyloric regions.

The distribution pattern of endocrine cells, which is predominant in the basal portion of oxyntic glands and spread over the pyloric glands, was not affected by vagotomy. On the other hand, the frequencies of endocrine cells in vagotomized calves were generally lower than control calves of the same age. The frequency of endocrine cells showed various reactions to vagotomy depending on endocrine cell types when the time course after vagotomy is considered. The number of immunorective endocrine cells in the lesser curvature was more affected by vagotomy than in the greater one, and more in the pyloric than in the corpus region. These tendencies might be associated with the innervation pathway along which the branches of vagus nerves run into the abomasal wall [10, 11].

Histamine-containing enterochromaffin-like (ECL) cells in addition to endocrine cell types investigated in the present study might be involved in chromogranin-IR cells in the corpus region [7, 25]. This cell type is reported to be sensitive to vagotomy [12]. The behavior of ECL cells possibly affects the frequency of chromogranin-IR cells in vagotomized calves. Gastrin-IR cells decreased at 3 weeks old in vagotomized calves and, in turn, recovered to the level of control calves at 5 weeks old. Serotonin- and somatostatin-IR cells decreased in the pyloric region of the lesser curvature of vagotomized calves as compared with control. However, the difference was not so conspicuous in the another regions. The frequencies of chromogranin- and gastrin-IR cells are likely to be more affected by vagotomy than those of serotonin- and somatostatin-IR cells. This may be associated with the regulatory roles of ECL and gastrin cells on gastric acid secretion from parietal cells [5, 12].

It is suggested that changes in the gastrointestinal neuroendocrine system after vagotomy varied, depending on the vagotomy methods used, such as partial or total and both or unilateral, and the interval after vagotomy [6, 21].

The present study demonstrated that truncal total vagotomy has a significant influence on the endocrine system of the abomasum. Furthermore, the change varied depending on the endocrine cell type and the period after vagotomy. This suggests that the endocrine system as well as the nervous system are important components of the intrinsic regulatory systems in the abomasum and might be disturbed by a malfunction of the vagus nerve.

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