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An immunohistochemical study on the distribution of endocrine cells in the digestive tract of gray goose (*Anser anser*)

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Abstract: The objective of this study was to investigate the morphology and the distribution of 5-hydroxytryptamine (5-HT), somatostatin (SS), gastrin (Gas), glucagon (Glu), and substance P immunoreactive (IR) cells in the digestive tract of gray goose by the immunohistochemical streptavidin-peroxidase method. The samples were taken from 10 healthy adult gray geese. The results showed that 5 kinds of IR cells were mainly distributed between the mucous epithelium and intestinal gland. The number of 5-HT-IR cells was highest in the rectum and duodenum, but none were observed in the pylorus. SS-IR cells appeared in great numbers in the pylorus, duodenum, and cecum; however, they were not found in esophagus. Gas-IR cells were mainly distributed in the glandular stomach and jejunum. Glu-IR cells appeared in small numbers in the glandular stomach, duodenum, and jejunum, but were not detected in other tissues. Substance P-IR cells were located in the jejunum, cecum, and rectum. Analysis of the present study showed that the distribution and morphological features of these 5 different endocrine cells were related to the feeding habits and metabolism in the digestive tract of the gray goose.

Key words: Gray goose, digestive tract, endocrine cells, immunohistochemistry

Introduction

During their long evolution, birds have developed a number of structural features that differ from those found in mammals; however, the distribution of endocrine cells in the gastrointestinal tract is related to species, living environment, feeding habits, and developmental status rather than evolution. Since the immunohistochemical method was first applied to endocrine cells in the digestive tracts of poultry by Larsson et al. (1) and Polak et al. (2), more than 10 types of endocrine cells have been detected in the avian digestive tract (3,4). Yamada et al. (5) used 6 antihormone sera to detect 7 kinds of endocrine cells in the glandular stomach of fowls; these cells were also widely distributed in the pylorus of the brown-eared pheasant (6). These endocrine cells were found in other waterfowl, such as Peking duck (7) and goose (8).

The main gastrointestinal hormones include 5-hydroxytryptamine (5-HT), gastrin (Gas), somatostatin (SS), glucagon (Glu), and substance P. An important neurotransmitter, 5-HT is able

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to stimulate smooth muscle contraction and vasodilation, which has a strong regulatory role in digestive function. There were 5-HT immunoreactive (IR) cells in the esophagus of amphibians and reptiles, but they were not detected in bird esophagi by El-Salhy et al. (9) or Tang and Deng (10). In the gastrointestinal tract of the white ibis, 5-HT-IR cells were mainly distributed in the duodenum, cecum, and rectum; Liu et al. (11) proposed that secretion of 5-HT in the hindgut may regulate bowel movements in order to facilitate excretion. Li et al. (12) demonstrated that the density of 5-HT-IR cells was more abundant in the rectum than in other regions of the intestinal tract; however, they were not detected in the esophagus, crop, or muscular stomach. SS is a hormone that has an antagonist impact with growth hormone. It is widely distributed in the nervous system, thyroid, gastrointestinal tract, pancreas, and other parts of vertebrates (13). Deng et al. (7) found that SS-IR cells in Peking duck were mainly distributed in the glandular and muscular stomach and reached the maximum density in the pylorus; they were only occasionally detected in the duodenum. In the digestive tract of *Gorsachius magnificus*, the density of SS-IR cells was greater in the foregut than in the midgut, and the cells were not detected in the hindgut (14). Gas stimulates gastric acid secretion and gastrointestinal motility, and it also accelerates gastric circular muscle contraction. In the digestive tract of the adult goose, Deng et al. (8) detected Gas-IR cells mainly in the muscular stomach, glandular stomach, and small intestine. Wang et al. (14) found no Gas-IR cells in the digestive tract of *Gorsachius magnificus*. The numbers of Glu-IR and substance P-IR cells in the digestive tract are lower than the numbers of 5-HT-IR and SS-IR cells. Liu et al. (11) found that substance P-IR cells were sparsely distributed in the digestive tract of the white ibis. In *Sphenomorphus indicus*, a reptile, Glu-IR cells were distributed only in the ileum and rectum, and substance P-IR cells were only detected in the mucosa epithelium of rectum; they were elliptical or spindle-like in shape (15). Shi et al. (16) observed the distribution of substance P-IR cells among the gastric epithelial cells of *Paralichthys olivaceus*. These cells were dissimilar in shape and size, but most of them were long rods or rotund, with one end stretching toward the lumen. As these studies illustrate, the distribution of endocrine cells in the digestive tracts of birds is different from that of mammals, and interspecific variety also exists.

Wild gray goose (*Anser anser*) is migratory and highly gregarious. It feeds on grass, which enables large flocks after domestication. Domesticated birds sexually maturate at 8-9 months of age, and the incubation period is 31 days. Gray goose meat, which is delicious and nutritious, is often regarded as a health product. Gray goose meat can be expensive, and raising these birds has high earning potential. Currently, there is no domestic or international study on the location of endocrine cells in the digestive tract of the gray goose.

In the present study, through immunohistochemical methods with 5 antisera, we studied the locations of 5-HT-IR, SS-IR, Gas-IR, Glu-IR, and substance P-IR cells in the digestive tract of the gray goose. The study was designed to reveal the rules and features of endocrine cell distribution and provide histological reference for further study of the functional mechanism of gastrointestinal hormones, the pathogenesis of gastrointestinal diseases, and the domestic breeding of the gray goose.

Materials and methods

Tissue specimens and reagents

The tissues were obtained from 10 healthy adult gray geese, 5 males and 5 females. Antiserum, poly-L-lysine (ZLI-9005), phosphate buffered saline (PBS), a DAB color system (ZLI-9032), and an SP-9000 immunohistochemistry kit were purchased from Zhong Shan Golden Bridge Biotechnology, Inc. (Beijing, China).

Methods

Tissue samples were collected immediately after euthanasia from the terminal esophagus, glandular stomach, muscular stomach, pylorus, duodenum, jejunum, ileum, cecum, and rectum and fixed in 4% paraformaldehyde for 6-24 h. The 5-μm-thick serial sections were prepared, dewaxed with xylene, and hydrated with ethanol and distilled water. The sections were soaked in PBS for 5 min and incubated in 3% hydrogen peroxide in absolute methanol for 10 min to block the endogenous peroxidase

activity. After incubation with normal goat serum for 15 min, the sections were incubated with the primary polyclonal antibody for 2 h. The universally biotinylated secondary antibody was added and incubated for 15 min. The sections were then incubated with streptavidin-peroxidase reagent for 15 min. Labeling was revealed by incubation with diaminobenzidine (DAB) for 5-10 min. Thorough washes were performed between steps, and all dilutions were carried out in PBS unless otherwise specified. All steps were performed at room temperature unless otherwise specified. Sections were counterstained with hematoxylin solution for 1 min. Finally, sections were dehydrated, cleared in xylene, and mounted with neutral gum. The labeling procedure for negative control sections was the same as described above, but PBS was substituted for the primary antibody. The sections were observed, and photographs were taken under a Leica DM2000 microscope.

Data processing and statistical analysis

Each tissue sample was processed by immunohistochemistry with 5 kinds of antiserum (5-HT, SS, Gas, Glu, and substance P). For each tissue, 5 slices were randomly chosen, 10 high-powered vision fields were randomly selected from each slice, and cells were counted. Data were shown as the average cells per vision field, and the ANOVA of SPSS 16.0 was applied to determine the significance of differences. Contrasts among the means of tissues were evaluated by Duncan's multiple range test at a significance level of $P < 0.05$.

Results

The IR cells for the 5 antibodies in the digestive tract of the gray goose appeared tawny and brown. The distribution of endocrine cells and their frequency are shown in the Table.

5-HT-IR cells

The 5-HT-IR cells were distributed widely in the digestive tract of the gray goose; they were round, elliptical, or conical with tawny staining. The cells were mainly scattered among the mucosal epithelium, intestinal gland epithelium, and acinus. The cells were predominant in the rectum (Figure 1). Most of them were round and a few were conical. The second highest average number of these cells was found in the duodenum. The density of the 5-HT-IR cells was similar in the ileum, jejunum, glandular stomach, and cecum. The cells were detected at low frequencies in the muscular stomach and the terminal esophagus. No 5-HT-IR cells were found in the pylorus. In the esophagus, 5-HT-IR cells were mainly localized in the mucosal epithelium and lamina propria. The cells were primarily observed in the gastric gland of the stomach region. In the duodenum, they were situated in the base of the intestinal villus and duodenal gland; jejunal and ileal localizations of the 5-HT-IR cells were restricted to the intestinal glands.

Table. Distribution of the endocrine cells in the digestive tract of gray goose (cells per 400× visual field).

	5-HT	SS	Gas	Glu	Substance P
Esophagus	1.80 ± 0.37^{c}	-	-	-	-
Glandular stomach	4.25 ± 0.25^{b}	2.40 ± 0.36^{c}	5.80 ± 0.76^{a}	2.20 ± 0.26	-
Muscular stomach	2.40 ± 0.51^{c}	1.83 ± 0.28^{c}	2.80 ± 0.38^{b}	-	-
Pylorus	-	4.60 ± 1.22^{a}	-	2.46 ± 0.60	-
Duodenum	6.00 ± 0.70^{a}	4.33 ± 0.98^{a}	2.12 ± 0.22^{b}	2.18 ± 0.82	-
Jejunum	4.25 ± 0.37^{b}	1.84 ± 0.66^{c}	5.44 ± 0.50^{a}	2.15 ± 0.12	1.95 ± 0.68
Ileum	4.83 ± 0.66^{b}	3.16 ± 0.32^{b}	-	-	-
Cecum	3.83 ± 0.47^{b}	4.16 ± 0.78^{a}	-	-	1.35 ± 0.85
Rectum	6.66 ± 0.71^{a}	3.33 ± 0.62^{b}	-	-	1.88 ± 0.24

Note: Values with different superscript letters represent significant differences ($P < 0.05$).

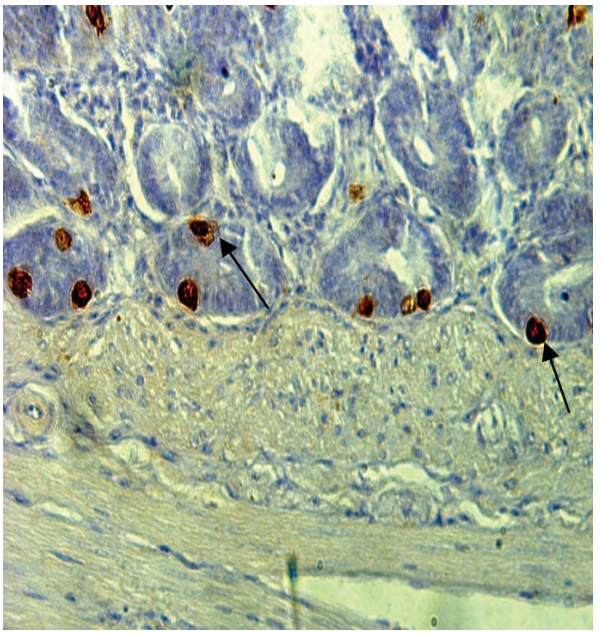

Figure 1. Cells containing 5-HT (arrows) in the rectum of gray goose (*Anser anser*), 400×.

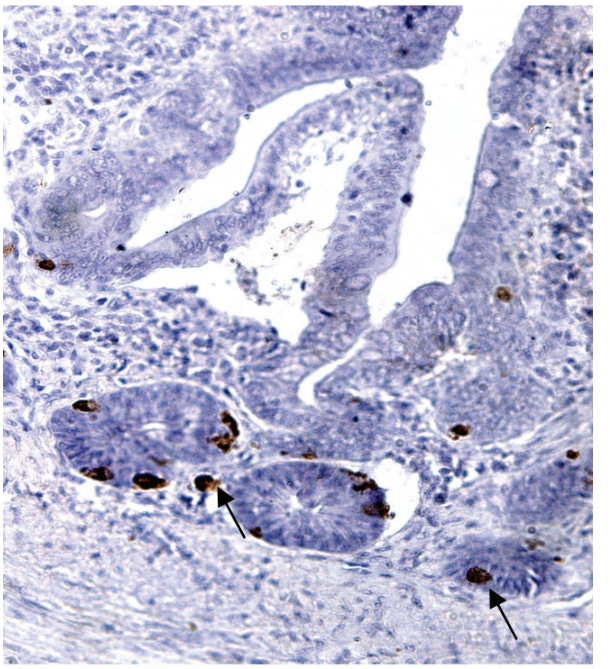

Figure 2. Cells containing SS (arrows) in the cecum of gray goose (*Anser anser*), 400×.

SS-IR cells

In the digestive tract of the gray goose, SS-IR cell distribution was inferior to 5-HT-IR distribution; the majority of SS-IR cells were round or elliptical while a few were cone-shaped, and the staining was brown. In the stomach, the SS-IR cells were primarily distributed between the glandular epithelium and mucous epithelium. In the duodenum, they were localized in the epithelium of the intestinal glands. In the jejunum, ileum, cecum, and rectum, they were mainly observed in the mucosal epithelium of the intestine. SS-IR cells were found more frequently in the pylorus. The density of SS-IR cells was similar in the duodenum and cecum (Figure 2). The cells were smaller in number in the glandular stomach, muscular stomach, and jejunum. SS-IR immunoreactive cells were not found in the esophagus.

Gas-IR cells

Gas-IR cells were less common than 5-HT-IR and SS-IR cells. They were located mainly in the glandular stomach, muscular stomach, jejunum, and duodenum. The staining was darker, and most of the cells were elliptical and scattered between the mucous and glandular epithelium. The Gas-IR cells were more frequently observed in the glandular stomach (Figure 3). The number of Gas-IR cells was the same in the jejunum and glandular stomach, and the cells were mostly elliptical or irregularly shaped. Gas-IR cells were occasionally observed in the muscular stomach and duodenum.

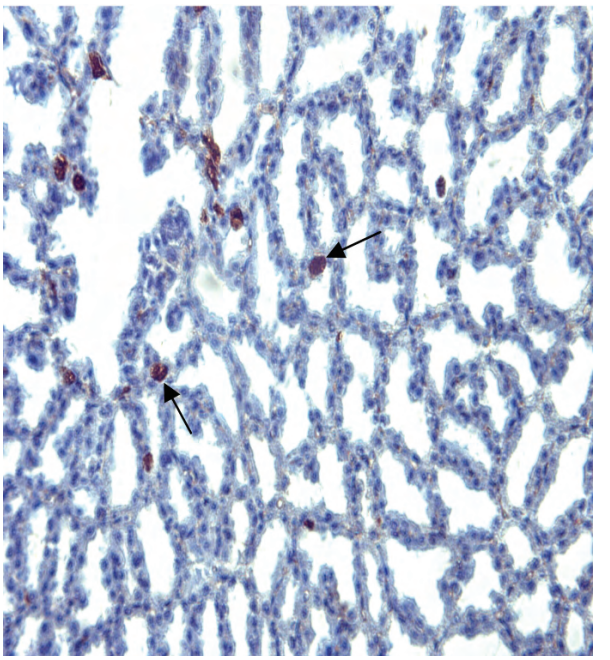

Figure 3. Cells containing Gas (arrows) in the glandular stomach of gray goose (*Anser anser*), 400×.

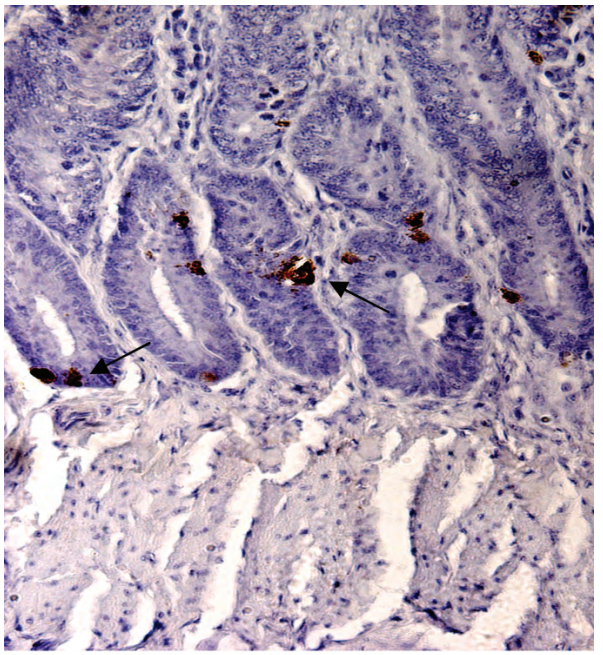

Figure 4. Cells containing Glu (arrows) in the duodenum of gray goose (*Anser anser*), 400×.

Glu-IR cells

Glu-IR cells were detected in the glandular stomach, pylorus, duodenum, and jejunum (Figure 4). They were round or cone-shaped and their staining was tawny. Glu-IR cells were predominantly located in the epithelium of the pyloric mucosa. Moderate numbers of Glu-IR cells were usually found in the glandular stomach, duodenum, and jejunum; the cells were sparsely distributed among the glandular epithelium.

Substance P-IR cells

The least commonly observed endocrine cell type among the 5 cell types was substance P-IR. An unspecific distribution pattern of endocrine cells in the digestive tract of the gray goose was also found for substance P-IR cells located between the mucous and glandular epithelium in the jejunum, cecum, and rectum. The staining was brown, and the cells were mostly round or elliptical; few were irregularly shaped. Substance P-IR cells were most numerous in the jejunum (Figure 5).

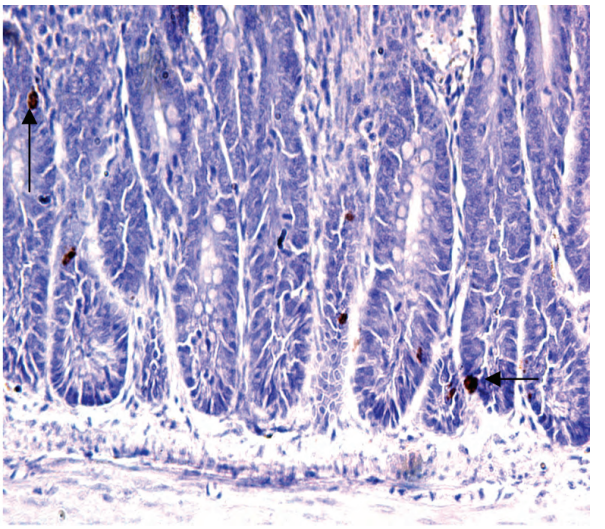

Figure 5. Microphotograph showing IR cells for substance P in the jejunum of gray goose (*Anser anser*). Arrows mark cells containing substance P; 400×.

Discussion

The widely distributed and numerous enterochromaffin cells (EC cells) were responsible for most of the 5-HT secretion, and both 5-HT and its receptors were involved in the mediation of gastrointestinal movement and secretion. Such findings have made EC cells a new research focus. EC cells were very sensitive to intestinal pressure and chemical stimulation and released 5-HT once intestinal pressure rose or the sugar that inhibits gastric emptying and regulates intestinal secretion appeared (17,18). Yang and Lackner (19) reported that EC cells had a synergistic effect with collaborative T cells and mast cells of the intestinal mucosa through the mechanism of immunization. This provides a morphological basis for research on the interaction of 5-HT with lymphocytes and indicates that 5-HT plays an important role in mucosal immunization. The birds do not store manure (20), and the muscles of the rectum need to contract rapidly to accommodate this physiological feature. The 5-HT stimulates smooth muscle contraction and consequently has a strong relationship with the regulation of excretion. The results obtained from this study showed that 5-HT-IR cells were located predominantly in the rectum of the gray goose; cell numbers in the rectum were significantly different than numbers from other studied locations. This is consistent with the function of the rectum. In addition, Zhang et al. (21) and Liu et al. (22) found few cone-shaped 5-HT-IR cells in the rectum of the gray goose with the cone top pointing toward the bowel antrum. It was speculated that the cells had both endocrine and exocrine functions. Li

et al. (23) reported that there were no 5-HT-IR cells in the esophagi of birds because food was temporarily stored and softened by the longer esophagi and 5-HT was unnecessary. According to the results of the present study, 5-HT-IR cells are scattered sparsely in the terminal esophagus in the gray goose, probably due to long-term artificial feeding. The number of 5-HT-IR cells in the intestinal tract was significantly different than the numbers in the muscular stomach; this was consistent with the results from 40-week-old HBK-SPF ducks (24). The different distributions of 5-HT-IR in different animals were ascribed to interspecific difference and might also be related to the features of digestion and metabolism (25-27).

SS was released by D-cells of the digestive tract and had a strong inhibitory effect on gastric acid secretion and gastrointestinal peristalsis. The presence of few or no SS-IR cells might be conducive for 5-HT to play its physiological role in the gastrointestinal tract and then regulate digestion and absorption of food in the digestive tract. As shown in the Table, SS-IR cells appeared in the highest numbers in the pylorus of the gray goose, while no 5-HT-IR cells were detected. This suggests that SS has an antagonistic impact on 5-HT. The number of SS-IR cells in the cecum was not significantly different from numbers found in the pylorus and duodenum, but significantly different from numbers detected in other tissues. This indicates that the function of the cecum in the gray goose is not obvious. Apart from the pylorus and cecum, SS-IR cells in the digestive tract of the gray goose were less numerous than 5-HT cells; this supports the view that the gray goose has strong digestion. The density curve was wavelike from the small intestine to the large intestine and reached its maximum number in the duodenum, which was significantly different from mammalian findings (28). No SS-IR cells were detected in the esophagus, which was consistent with the immunolocalization results in the Zi goose, *Takydromus wolteri*, and pigs (21,23,29).

Gas was secreted by G-cells localized in the mucosa of the stomach and small intestine and was released because of the mechanical action of gastric contents on G-cells. Gas had the ability to strongly stimulate the secretion of gastric acid, promote the contraction of the pyloric sphincter, and accelerate cell proliferation in the stomach and small intestine. Gas-IR cells in the glandular stomach and jejunum of the gray goose were significantly greater in number than those in the muscular stomach and duodenum; this indicates that the epithelium of the glandular stomach and jejunum have a strong proliferation ability. The smaller number of Gas-IR cells in the muscular stomach might be due to the artificially fed gray geese used in this study. The Gas-IR cells in the duodenum and jejunum play an important role in the absorption of nutrients. These results were significantly different from those from other geese, *Gorsachius magnificus*, and mammals (14,23,25).

Glu is a single-chain peptide of 29 amino acid residues secreted by α-cells. When the nutrient supply is inadequate or the metabolism needs enhancing, Glu can activate phosphorylase and lipase to promote the decomposition of glycogen and fat and reduce the advance speed of chyme in the intestinal tract. Glu-IR cells were distributed in great number in the pylorus and in the glandular stomach, duodenum, and jejunum with no significant differences; this may help the gray goose endure hunger and lack of food.

Substance P was the earliest discovered neuropeptide, an important messenger substance for transmitting information and regulating organism response (30). Substance P can also accelerate the contraction of smooth muscle in the large intestine, stimulate secretion of the goblet cells, and help produce smooth excretion. In the present study, there were only a tiny number of substance P-IR cells in the digestive tract of the gray goose, and these were sparsely distributed in the jejunum and large intestine with no significant difference noted between them. This would help convert food into chyme and enhance the rates of digestion and absorption. In addition, the secretion of substance P had a synergistic effect on 5-HT, which was consistent with the results in adult mammals (23).

Although there are dozens of kinds of endocrine cells in the digestive tract, this study focused on 5 of them. The relationship between endocrine cells and active substances, the seasonal impact on the distribution and secretion of endocrine cells, and a comparison of endocrine cell distribution in other bird species are important topics for future investigation.

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