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Short communication

Immunohistochemical study of endocrine cells in the stomach and small intestine of the lion (*Panthera leo*)

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Abstract. The distribution and relative frequency of gastrointestinal endocrine cells were investigated in the stomach and small intestine (duodenum and jejunum) of a lion (*Panthera leo*). The present study was an immunohistochemical investigation of endocrine cells showing immunoreactivity for serotonin, gastrin, or cholecystokinin (CCK). Serotonin-immunoreactive (IR) cells were detected throughout the gastrointestinal tract with the highest frequency in the duodenum. Gastrin-IR cells were numerous in the pyloric region of the stomach and moderate numbers were also seen in the duodenum and jejunum. Moderate numbers of CCK-IR cells were only detected in the duodenum and jejunum.

Key words: endocrine cells, lion, small intestine, stomach.

The mucosal epithelium of the alimentary tract (stomach and intestines) and the pancreas not only includes exocrine cells, but also endocrine cells that produce hormones, and these endocrine cells form the gastroenteropancreatic endocrine system (Fujita et al. 1988; Sundler and Håkanson 1988). The gastrointestinal tract is considered to be the largest endocrine organ in the body, because it contains endocrine cells widely distributed from the cardia to the rectum (Thompson 1987; Sundler and Håkanson 1988). Endocrine cells of the gastrointestinal tract have been classified by shape, size, internal structure, argentaffin staining, electron density of the secretory granules containing hormones, and/or by immunohistochemical staining (Grube and Forssmann 1979; Solcia et al. 1987, 1989). The hormones secreted by various endocrine cells act as chemical messengers to regulate gut functions.

Many studies have investigated the regional distribution and relative frequencies of various endocrine cells in the gastrointestinal tracts of domestic mammals (e.g., Lebedeva and Levedev 1972; Kitamura et al. 1982, 1984, 1985; Ito et al. 1987; Ceccarelli et al. 1995a; Galán et al. 1996; Chen and Zhang 2008) and laboratory rodents (e.g., Pinto et al. 1995; Spangeus and El-Salhy 1998; Spangeus et al. 1999; Ku et al. 2002, 2006). Moreover, studies of gastrointestinal endocrine cells performed in many wild animals have been reported, including marsupials (e.g., koalas, great grey kangaroos, Parma wallabies, tiger cats, and opossums) (Krause et al. 1985; Yamada et al. 1987; Takagi et al. 1990), monotremes (platypuses and echidnas) (Yamada and Krause 1983; Yamada et al. 1985), sloths (Mota et al. 1992), musk shrews (Kitamura et al. 1990), tree shrews (Yamada et al. 1999), camels (Eerdunchaolu et al. 2001), fallow deer (Ceccarelli et al. 1995b), lesser mouse deer (Agungpriyono et al. 1994), barking deer (Adnyane et al. 2011), babirusas (Agungpriyono et al. 2000), wild boars (Dall'Aglio et al. 1998), bats (Nisa et al. 2000), and wolves (Chen and Zhang 2008).

The lion (*Panthera leo*), which belongs to the family

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Felidae, the suborder Feliformia, the order Carnivora, shows an obligate flesh-eating and hunts the preys such as wildebeests (gnus), impalas, giraffes, buffalo, and zebra (Nowak 1999). It has been reported that the ratio of intestine to body length of the cat (obligate flesh-eating), 4:1, is lower than that of the dog (flesh-eating with case omnivorousness), 6:1, and this ratio is much higher in herbivores because of the lower digestibility of diet; horse, ox, and sheep is 12:1, 20:1, and 27:1, respectively (Hamper 2015). The gastrointestinal tract of lions is relatively simple, short, and uncomplicated, and the intestinal length without caecum in adult lions is approximately 7.5 m (average, n = 3) (Smith et al. 2006). The average body length of lions, reported by Hollister (1918), is about 1.7 m (n = 10). The ratio of intestine to body length of the lion was, thus, 4.4. It may be assumed that this lower ratio is one of the morphological features of the obligate carnivorous digestive tract. It would be valuable to examine the physiological characteristic such as hormonal synthesis in obligate carnivores with shorter gastrointestinal tract and to compare it with that of mammals with other feeding habits.

The present study aimed to immunohistochemically investigate the regional distribution and relative frequency of endocrine cells secreting gastrointestinal hormones, such as serotonin (5-hydroxytryptamine, 5-HT), gastrin, and cholecystokinin (CCK), in the stomach and small intestine of the lion, which is an obligate carnivore.

Materials and methods

Animal and sample preparation

The carcass of a male lion that died of squamous cell carcinoma in 2011 at 19 years old was donated by the Obihiro zoo (Obihiro, Hokkaido, Japan). The metastasis of tumor to the digestive tract was not confirmed by pathological diagnosis. Tissue samples were taken from the stomach (cardiac, oxyntic, and pyloric regions), duodenum (proximal region), and jejunum (mid-region) and fixed in Bouin's solution for 24 h. Then the samples were transferred to 70% ethanol, dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin (Paraplast Plus®, Kendall, MA, USA). Tissues were cut into sections 4 μm thick that were mounted on gelatin-coated slides.

Immunohistochemistry

For immunohistochemical staining, the sections were deparaffinized in xylene, rehydrated in decreasing concentrations of ethanol, and then rinsed in running water. Endogenous peroxidase activity was blocked by incubation with 0.3% H_2O_2 in methanol at room temperature (RT) for 10 min. Then the samples were washed three times with 0.01 M phosphate-buffered saline (PBS, pH 7.4), and incubated in normal goat serum (1:50, S-1000, Vector Laboratories Inc., CA, USA) for 30 min at RT. The sections were again washed with PBS, and incubated with one of the primary antisera (Table 1) at 4°C overnight. On the second day, the sections were washed in PBS and incubated at RT for 30 min with the secondary antibody: biotinylated goat anti-rabbit IgG (1:200, BA-1000, Vector Laboratories Inc.) or biotinylated goat anti-guinea pig IgG (1:200, BA-7000, Vector Laboratories Inc.). After further washing with PBS, the sections were incubated with avidin-biotin-peroxidase complex (PK-6100, Vectastain *Elite* ABC kit, Vector Laboratories Inc.). Reaction products were visualized by incubation with 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) and 0.006% H_2O_2 in Tris buffer (pH 7.4). Then the sections were lightly counterstained with Mayer's hematoxylin, dehydrated in ascending concentrations of ethanol, cleared in xylene, and cover-slipped. Immunoreactive (IR) cells were observed under a conventional light microscope and the frequency of each IR cell type was classified subjectively into four grades: (–) not detected, (+) few, (++) moderate, and (+++) numerous.

Results and discussion

In the present study, three types of endocrine cells showing immunoreactivity for serotonin, gastrin, or CCK were detected in the mucosal epithelium of the gastrointestinal tract with different distributions and frequencies (Table 2). Serotonin-IR cells were found at low to moderate numbers in all regions of the stomach and the jejunum (Fig. 1A), while there were numerous serotonin-IR cells in the duodenum (Fig. 1B). Gastrin-IR cells were not detected in the cardiac and oxyntic regions of the stomach, but were frequent in the pyloric region (Fig. 1C) and showed moderate numbers in the small intestine. CCK-IR cells were only found in the small intestine where moderate numbers were detected (Fig. 1D).

In the present study, the regional distribution and frequency of endocrine cells were investigated in the stomach and small intestine of the lion. There was variation in the frequency of the endocrine cells examined among different regions of the stomach and small intestine. It is

Table 1. Antibodies used in this study

Antibody	Host animal	Dilution	Code	Sources
Serotonin	Rabbit	1:10 000	Sero 2-3	Dr. Nishiitsutsuji-Uwo, Kyoto, Japan
Gastrin	Guinea pig	1:6000	GP-1304	Dr. Yanaihara, Shizuoka, Japan
Cholecystokinin	Rabbit	1:6000	RPN.1742	Amersham, Amersham, UK

Table 2. Regional distribution and frequency of endocrine cells in the stomach and small intestine of the lion

	Stomach			Small intestine	
	Cardiac	Oxyntic	Pyloric	Duodenum	Jejunum
Serotonin	+	++	+	+++	++
Gastrin	–	–	+++	++	++
Cholecystokinin	–	–	–	++	++

–: Not detected, +: Few, ++: Moderate, +++: Numerous.

generally accepted that the abundance of each endocrine cell type shows marked differences depending on the animal species and the region of the gastrointestinal tract (Solcia et al. 1975).

In many mammals, it has been demonstrated that serotonin-IR cells are widely distributed in the gastrointestinal tract, which contains about 95% of the serotonin in the whole body (Kitamura et al. 1985; Ito et al. 1987; Camilleri 2009). In the present study, serotonin-IR cells were detected throughout the stomach and small intestine, and showed a higher frequency in the duodenum compared with all regions of the stomach. Serotonin secreted from enterochromaffin (EC) cells mainly mediates the contraction (via excitatory cholinergic motor neurons or directly) or relaxation (via inhibitory nitrergic motor neurons or directly) of smooth muscle in the gastrointestinal tract (i.e., peristalsis) and regulates the synthesis of gastric and colonic mucus via various 5-HT receptor subtypes (5-$HT_{1–7}$ receptors) (Ormsbee and Fondacaro 1985; Gershon 2004; Sikander et al. 2009). In present study, the abundance of serotonin-IR cells in the duodenum of the lion suggests that secretion of serotonin is markedly stimulated by the food bolus entering the duodenum from the stomach, after which serotonin may induce mucus secretion and/or trigger peristalsis in the small intestine. In the stomach also, the immunoreactivity for serotonin was detected although the frequency was lower compared with the duodenum. It may be assumed that the secretion of serotonin in the stomach promotes the secretion of gastric mucus and the gastric motility. It is suggested that the functions of gastrointestinal serotonin might have been preserved among mammalian species because of its distributional similarity.

Gastrin and CCK both belong to the CCK-gastrin family and have a common evolutionary origin with same C-terminal sequence (Larsson and Rehfeld 1977; Rehfeld et al. 2007). It is well known that gastrin (mainly gastrin-17: G-17, little gastrin) secreted by pyloric G cells indirectly promotes gastric acid secretion by parietal cells via histamine secreted from enterochromaffin-like (ECL) cells, which express CCK_2 receptors stimulated by gastrin, and also has a lesser direct effect on gastric acid secretion via CCK_2 receptors expressed on parietal cells, as well as regulating the growth of mucosal epithelial cells in the fundus (Lamers et al. 1982; Guilloteau et al. 2006; Rehfeld et al. 2007). It has been reported that duodenal G cells synthesize and secrete not only G-17, but also gastrin-34 (G-34, big gastrin), at about the same level (Lamers et al. 1982; Walsh 1994; Guilloteau et al. 2006), although G-34 is less effective at stimulating gastric acid secretion than G-17 (Lamers et al. 1982). It has been demonstrated that a high proportion of circulating G-34 is secreted from the duodenum and that G-34 has a longer half-life compared with G-17 (Lamers et al. 1982; Rehfeld et al. 2007). Therefore, the gastrin secreted from the intestine constantly may regulate the growth and proliferation of the mucosa (Guilloteau et al. 2006; Rao and Wang 2011). In the lion, gastrin-IR cells were confirmed to have a high frequency only in the pyloric region, and were less abundant in the small intestine as other mammals previously examined. Regardless of feeding habits or phylogenetic classification, it may be assumed that the primary role of gastrin is shared between pyloric (promotion of gastric acid secretion) and duodenal (mucosal growth) G

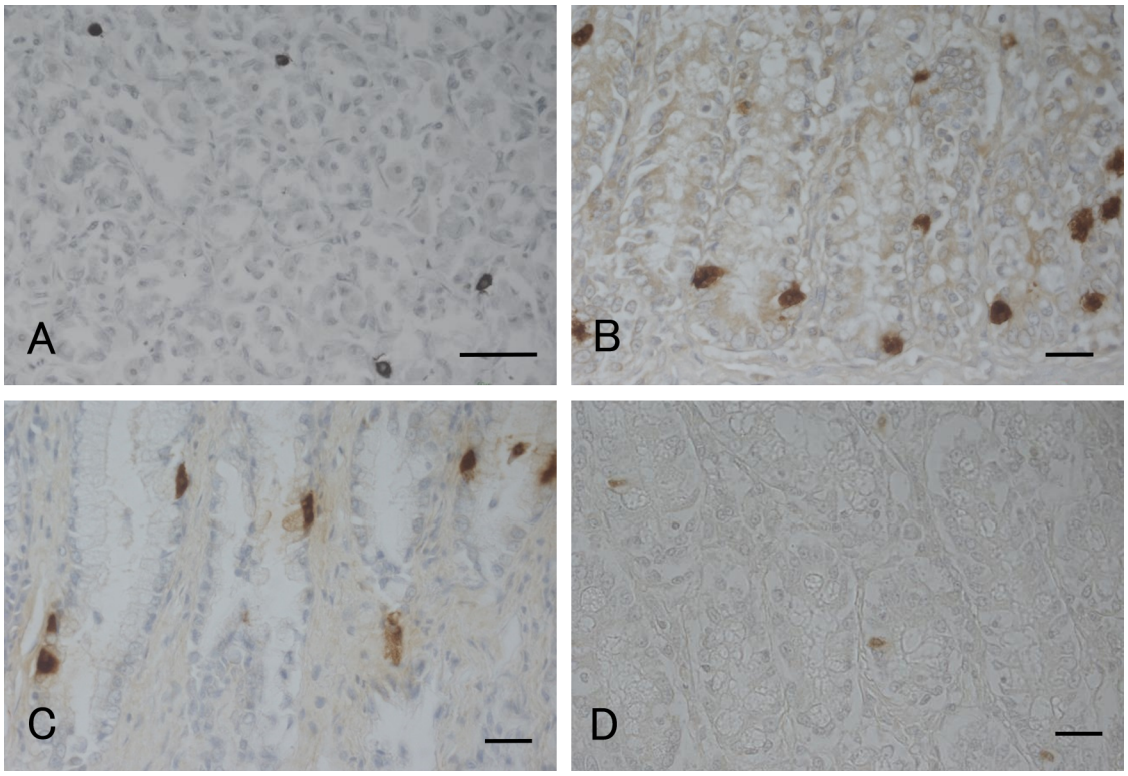

Fig. 1. Immunohistochemical observation of endocrine cells in the stomach and small intestine of the lion. A: serotonin-IR cells in the oxyntic region of the stomach, B: serotonin-IR cells in the duodenum, C: gastrin-IR cells in the pyloric region of the stomach, D: cholecystokinin-IR cells in the duodenum. Bar = 50 μm (A), 20 μm (B–D).

cells in the lion.

Cholecystokinin is secreted by I cells and acts via CCK_1 receptors (Guilloteau et al. 2006). It is well known that CCK has many important functions in the GEP system: 1) regulation of pancreatic enzyme secretion and growth; 2) regulation of gallbladder contraction and relaxation of the sphincter of Oddi (release of bile into the duodenum); 3) regulation of glucagon secretion; 4) inhibition of gastric acid secretion; 5) delay of gastric emptying; 6) promotion of intestinal motility; 7) promotion of small intestinal enzyme secretion; and 8) elevation of intestinal blood flow increase (Guilloteau et al. 2006; Rehfeld et al. 2007). The general pattern of distribution of CCK-cells is that these cells are absent in the stomach, decrease in numbers before the distal small intestine, and are absent in the large intestine. In the lion examined in the present study, moderate immunoreactivity for CCK was still detected in the jejunum unlike general pattern, and similar result was observed in cats also (Kitamura et al. 1982; Yamada 1985). In general, felids show obligate flesh-eating depending on prey meats and have shorter intestine. In carnivores, peptides and lipids entering the duodenum should be promptly digested by several enzymes and then absorbed in intestinal capillary vessels before excretion from the body because of shorter intestine. Therefore, this extended CCK-IR cells distribution may more persistently promote the secretion of pancreatic and small intestinal enzymes, the release of bile, and the uptake of degradation products into circulation. There have been some reports of gastrointestinal endocrine cells in other carnivore species such as dogs, wolves, minks except cats (Lebedeva and Levedev 1972; Kawano et al. 1983; Galán et al. 1996; Chen and Zhang 2008). However, these reports were not able to use for the comparison of the regional distribution and relative frequency of CCK-RI cells because of the absence of data for CCK.

The present study clarified the regional distribution and relative frequency of endocrine cells containing serotonin, gastrin, or CCK in the stomach and small intestine (duodenum and jejunum) of the lion, and the extended moderate distribution of CCK-IR cells was demonstrated in the jejunum. In the present study, we were not able to investigate the ileum and large intestine or more than one animal. In further studies, investigation of the entire gastrointestinal tract in a number of animals will be required to comprehensively understand the actions of

gastrointestinal hormones in the lion. Furthermore, it is essential for further discussion to examine the regional distribution and relative frequency of gastrointestinal endocrine cells in carnivore species with strong creophagy.

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