Immunohistochemical Survey of the Gut Endocrine Cells in the Common Tree Shrew (*Tupaia belangeri*)

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ABSTRACT. Regional distribution and relative frequency of endocrine cells in the gastrointestinal tract of the common tree shrew (*Tupaia belangeri*) were studied immunohistochemically. Ten types of immunoreactive endocrine cells were localized in the gastric mucosa, i.e., chromogranin-, serotonin-, gastrin-, somatostatin-, bovine pancreatic polypeptide (BPP)-, enteroglucagon-, pancreatic glucagon-, peptide tyrosine tyrosine (PYY)-, motilin-, and substance P (SP)-immunoreactive (IR) cells. In the intestine, 13 types of immunoreactive cells were observed, i.e., chromogranin-, serotonin-, somatostatin-, gastrin-, BPP-, enteroglucagon-, PYY-, secretin-, cholecystokinin (CCK)-, gastric inhibitory peptide (GIP)-, motilin-, neurotensin-, and SP-IR cells. The regional distribution and relative frequency of the cell types varied along the gastrointestinal tract. Basically, the types, distribution, and relative frequency of the gut endocrine cells were similar to those reported in other mammalian species. However, some characteristic findings were noted in the present study: (1) the considerably large number of gastrin-IR cells in the pyloric region; (2) numerous serotonin-IR cells in the stomach; (3) appreciable number of BPP-IR cells in the transitional region of the stomach; and (4) wide distribution of PYY- and motilin-IR cells in the gut.—**KEY WORDS:** gut endocrine cell, immunohistochemistry, intestine, stomach, tree shrew.

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Tree shrews, which resemble small long-nosed squirrels, belong to the Tupaiidae, the only family representing the order Scandentia. They exhibit some morphological characteristics common to rodents, insectivores and primitive primates. The complexity of their anatomical structure has raised controversy as to their exact taxonomic classification for many decades [25]. They have been previously classified either as insectivores [6, 33] or as primates [7, 26, 34, 35]. However, the current accepted classification is under an independent order, the Scandentia [4, 9, 29, 38].

The common tree shrew, *Tupaia belangeri* (*T. belangeri*) is the most abundant species under the order Scandentia. They are widely distributed in Southeast Asia and inhabit a wide range of arboreal, semi-arboreal, and forest floor niches [25, 26]. They are primarily insectivorous and frugivorous. Their gastrointestinal tract consists of simple stomach and short intestine with a small cecum [26, 28].

Numerous reports have been published on the gastrointestinal tract of many vertebrate species with special reference to their gut endocrine cells. For those under order Scandentia, studies have been concentrated to only one species, the *Tupaia belangeri*, and the scope has covered a few types of endocrine cells and limited region of the gut [12–14]. The present study was, therefore, conducted to clarify the regional distribution and relative frequency of occurrence of endocrine cells in the whole length of the

gastrointestinal tract of the common tree shrew, *T. belangeri*, using specific immunohistochemical method.

MATERIALS AND METHODS

Seven adult animals, *T. belangeri*, of both sexes (weighing 110–140 g) were used in the study. They were sacrificed by exsanguination under sodium pentobarbital anesthesia. Immediately after death, the whole gastrointestinal tract was taken and then fixed overnight in Bouin's fluid. To facilitate penetration, fixative was injected into the lumen. Tissue samples were collected from the different regions of the gastrointestinal tract (Fig. 1). The whole stomach, from cardia to pylorus, was taken and both lateral sides were sliced off. All the tissue blocks collected were processed routinely by embedding in paraffin. Serial sections were cut at 5 μ m using a microtome. They were deparaffinized and incubated with 0.3% H₂O₂ in methanol for 10 min. After washing in running water and 0.1 M phosphate buffered saline (PBS), pH 7.3, the sections were incubated with non-immunized goat or rabbit serum for 30 min at room temperature. They were immunostained using avidin-biotin peroxidase complex (ABC) method [16]. Incubation periods were as follows: (1) overnight at 4°C with primary antisera (Table 1); (2) 30 min with secondary antibody at 1:200 dilution (for gastrin; biotinylated anti-guinea pig IgG serum raised in goat, for substance P; biotinylated anti-rat

IgG serum raised in rabbit, and for the rest of the antisera; biotinylated anti-rabbit IgG serum raised in goat; Vector, Burlingame, U. S. A); and (3) 30 min at room temperature with Vectastain ABC kit (Vector). The immunoreaction was visualized using 3-3'-diaminobenzidine tetrahydrochloride-H₂O₂ solution in 0.03% w/v Tris-HCl buffer (Dojindo, Kumamoto, Japan).

The specificity of each immunoreaction was checked by

Fig. 1. Sampling portions in the gastrointestinal tract of the common tree shrew. 1=cardiac gland region; 2=transitional region between the cardiac gland region and pyloric gland region in the lesser curvature; 3=proper gastric gland region; 4=transitional region between the cardiac gland region and proper gastric gland region in the greater curvature; 5=pyloric gland region; 6=Brunner's glands in the proximal duodenum (7); 7=proximal duodenum; 8=middle region of proximal half of the small intestine (proximal jejunum); 9=middle region of the small intestine (middle jejunum); 10=middle region of distal half of the small intestine (distal jejunum); 11=ileum; 12=cecum; 13=proximal colon; 14=middle region of the large intestine (distal colon); 15=rectum.

replacement of specific antiserum with (1) normal serum, (2) PBS or (3) antiserum pre-absorbed overnight at 4°C with its corresponding antigen at <100 µg/ml antiserum as working dilution (Table 1). The endocrine cells immunoreactive for glucagon antiserum with code No. RPN 1602 were referred to as enteroglucagon-IR cells since the antiserum showed cross-reaction with pancreatic- and entero-glucagons. On the other hand, those which were immunoreacted using glucagon antiserum with code No. GL-5 were referred to as pancreatic glucagon-IR cells as it reacted specifically with pancreatic glucagon.

The relative frequency of occurrence, mean number and standard deviation of each type of immunoreactive cell per 1.0 mm² of microscopic field were determined using image-analyzing system (SPICCA, Nippon Avionics, Tokyo, Japan).

Another sets of deparaffinized sections were stained using hematoxylin-eosin and alcian blue-periodic acid Shiff (AB-PAS) for general observation.

RESULTS

Gastric endocrine cells: The gastric mucosa of *T. belangeri* was divided into 5 regions, viz., cardiac gland, proper gastric gland, pyloric gland, and 2 transitional regions. The transitional regions comprise the area between the cardiac and pyloric gland regions of the lesser curvature and the area between the proper gastric and pyloric gland regions of the greater curvature (Fig. 1). These transitional regions include the mucous neck, mucous and parietal cells with or without a few chief cells.

In the gastric mucosa, 10 types of endocrine cells were demonstrated (Table 2, Figs. 2a-d). They were immunoreactive for chromogranin, serotonin, gastrin, somatostatin, bovine pancreatic polypeptide (BPP), enteroglucagon, pancreatic glucagon, peptide tyrosine tyrosine (PYY),

Table 1. Antisera used to study gut endocrine cells in the common tree shrew

Antiserum target (code)	Working dilution	Source	Antigen used for preabsorption test Source (code)
Chromogranin (Lot 8541011)	1:10,000	Immuno Nuclear, Stilwater, OK., U.S.A.	NT
Serotonin (Lot 16302)	1:150,000	Immuno Nuclear	Sigma, St. Louis, MO., U.S.A. (H575, 111F-72652)
Gastrin (GP-1304)	1:5,000	N. Yanaihara, Shizuoka, Japan	N. Yanaihara (NY-FS-1-72)
Somatostatin	1:10,000	S. Ito, Niigata, Japan	Bachem, Torrance, CA., U.S.A. (R5574)
BPP (Lot 615-R110-146-17)	1:10,000	R. E. Chance, Indianapolis, IN., U.S.A.	R. E. Chance (Lot 615-D63-166-9)
Glucagon (GL-5)	1:60,000	N. Yanaihara	Lilly, Indianapolis, IN., U.S.A. (Lot 258-251-120)
Glucagon (RPN 1602)	1:5,000	Amersham, Amersham, U.K.	Lilly (Lot 258-251-120)
PYY (R-841303-2)	1:10,000	Milab, Malmo, Sweden	NT
Secretin (R 803)	1:2,000	N. Yanaihara	N. Yanaihara (MK-XII-F-II-3)
CCK (RPN 1742)	1:4,000	Amersham	Calbiochem, La Jolla, CA., U. S.A. (R4090)
GIP (G/R/34-IIID)	1:80,000	Guildhay, Guildford, U.K.	Bachem (R5375)
Motilin (R-1104)	1:8,000	N. Yanaihara	Bachem (R5720)
Neurotensin (R-3501)	1:10,000	N. Yanaihara	Bachem (R4322)
SP (MAS035b)	1:1,000	Sera-lab, Crawley Down, U.K.	Peptide Institute, Osaka, Japan (4014-V)

All antisera were raised in rabbits except those against gastrin and SP, which were raised in guinea pig and rat, respectively. BPP=bovine pancreatic polypeptide; PYY=peptide tyrosine tyrosine; CCK=cholecystokinin; GIP=gastric inhibitory peptide; SP=substance P; - = not applicable; NT=not tested.

Table 2. Regional distribution and relative frequency of gut endocrine cells in the common tree shrew

Endocrine cells	Stomach					Small intestine					Large intestine				
	1 ^{a)}	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Chromogranin	+++	+++	+++	+++	+++	+	+++	+++	+++	+++	+++	++++	+++	++++	+++
Serotonin	+++	++++	++++	+++	++++	+	+++	+++	+++	+++	++++	++++	+++	+++	+++
Gastrin	-	-	-	-	+++++	+	+++	++	+	+	-	-	-	-	-
Somatostatin	+	+	+++	+	+++	-/+	+	+	+	+	+	-/+	-/+	-/+	-/+
BPP	-	++	++	+++	+	+	+	-/+	-/+	-/+	-	-	-	-/+	-
Enteroglucagon	+	-/+	+++	-	-	-/+	-/+	+	+	++	+	+	+	-/+	+
Pancreatic glucagon	-	-	+++	-	-/+	-	-	-	-	-	-	-	-	-	-
PYY	-/+	+	-/+	-	+	+	-/+	-/+	-/+	+	+	++	++	++	+
Secretin	-	-	-	-	-	-	+++	+	-/+	-	-	-	-	-	-
CCK	-	-	-	-	-	-	+	-/+	-	-	-	-	-	-	-
GIP	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-
Motilin	-	-	-	-	-/+	-/+	++	+	+	+	+	-/+	+	+	-/+
Neurotensin	-	-	-	-	-	-/+	-/+	+	++	++	+	-	-	-	-
SP	-	+	+	-/+	-/+	-	+	+	+	+	++	+	+	-/+	-/+

a) Arabic numerals show segments of gastrointestinal tract corresponding to those in Fig. 1. - = not detected, -/+ = detected but < 1.0 cells/mm², + = 1.0–9.9 cells/mm², ++ = 10.0–19.9 cells/mm², +++ = 20.0–49.9 cells/mm², ++++ = 50.0–100 cells/mm² and +++++ = > 100 cells/mm².

motilin, and substance P (SP). They were localized mainly in the glandular body and base. They were round, oval or pyramidal in shape (Figs. 2a–d). Some were open-type cells having luminal contact with their apical cytoplasmic processes. In the proper gastric glands, however, the endocrine cells were primarily round or oval in shape with no open-type cells detected, but only occasional cells with cytoplasmic processes running along the basal lamina.

Among the 10 types of gastric immunoreactive endocrine cells, only chromogranin-, serotonin-, and somatostatin-immunoreactive (IR) cells were localized in all parts of the gastric mucosa. The rest showed restricted distribution. Serotonin-IR cells were the most abundantly distributed cell type with a frequency of approximately 50–100 cells/mm² in all the regions of the stomach. Chromogranin-IR cells displayed a fairly uniform moderate frequency at 20–50 cells/mm² throughout the gastric mucosa. Somatostatin-IR cells were moderate in number in proper gastric and pyloric gland regions, and a few in other regions of the stomach. BPP-IR cells were moderate in the transitional region between the cardiac and pyloric gland regions of the greater curvature, and a few in other regions except in the cardiac gland region where they were undetected. Gastrin- and motilin-IR cells were only detected in the pyloric gland region. However, the former were at exceedingly large number while the latter were occasionally observed. Enteroglucagon- and pancreatic glucagon-IR cells were both moderate in number in the proper gastric gland region. In the cardiac gland region, a few enteroglucagon- and somatostatin-IR cells, and scarce PYY-IR cells were observed. The proper gastric gland region showed a few SP- and scarce PYY-IR cells while the pyloric gland region consisted of a few PYY-, and scarce pancreatic glucagon- and SP-IR cells. In the transitional region between the cardiac and pyloric gland regions of the lesser curvature, a

few PYY- and SP-, and rare enteroglucagon-IR cells were noted, while in the transitional region between the cardiac and proper gastric gland regions of the greater curvature, occasional SP-IR cells were demonstrated.

Intestinal endocrine cells: Thirteen types of endocrine cells immunoreactive for chromogranin, serotonin, somatostatin, gastrin, BPP, enteroglucagon, PYY, secretin, cholecystokinin (CCK), gastric inhibitory peptide (GIP), motilin, neurotensin and SP were found in the intestine (Table 2). In the intestinal villi, endocrine cells were long- or short-spindle or pyramidal in shape (Figs. 2e–j). Generally, they were somewhat more numerous in the intestinal crypts than in the intestinal villi. The open-type endocrine cells were found frequently along the entire intestine (Figs. 2f–h).

The distribution and frequency of 13 endocrine cell types varied with cell type and intestinal segment (Table 2). Each of them had different distribution pattern and frequency of occurrence (Table 2). Endocrine cells immunoreactive for chromogranin, serotonin, somatostatin, enteroglucagon, PYY, motilin and SP were detected in the mucosal epithelium along the entire length of the intestine. Among them, serotonin-IR cells were the most numerous endocrine cells in the intestine especially in the ileum and cecum (about 75 cells/mm²). Chromogranin-IR cells were also numerous (over 30 cells/mm²) along the intestine except in Brunner's gland. They increased in number to about 74 cells/mm² in the cecum and proximal colon. Gastrin-, secretin-, motilin- and GIP-IR cells were predominant in the proximal small intestine and their numbers decreased towards the ileum. Gastrin-, secretin-, and motilin-IR cells were found most frequently in the proximal duodenum (about 27, 20 and 17 cells/mm², respectively). A small number of GIP-IR cells were found in the whole small intestine but predominant in the distal duodenum (about 8 cells/mm²). CCK-IR cells were distributed only in the

Fig. 2. Microphotographs showing immunoreactive gut endocrine cells in the gastrointestinal mucosa of the common tree shrew (ABC method, $\times 480$). (a) Relatively numerous serotonin-IR cells in the transitional region from the proper gastric gland region to pyloric gland region in the greater curvature. (b) Very numerous gastrin-IR cells in the basal portion of the pyloric gland. (c) Two BPP-IR cell in the glandular body in the transitional region from the proper gastric gland region to pyloric gland region of the greater curvature. (d) Four pancreatic glucagon-IR cells in the body of the proper gastric glands. (e) A PYY-IR cell in the intestinal villus of the proximal duodenum. (f) A secretin-IR cell in the intestinal villus of the proximal duodenum. This is an open-type cell having a luminal contact with apical cytoplasmic process. (g) An enteroglucagon-IR cell on the intestinal villus of the proximal jejunum. This is an open type. (h) A GIP-IR cell on the intestinal villus of the proximal jejunum. This cell is an open type. (i) Two motilin-IR cells in the intestinal crypt of the distal colon. (j) An SP-IR cell in the intestinal crypt of the ileum.

proximal (about 1 cell/mm²) and distal duodenum (about 0.5 cell/mm²). BPP-IR cells were found in all segments of the small intestine, while they were found rarely in the distal colon. Neurotensin-IR cells were found in all segments of the small intestine, but they were most numerous in the jejunum (about 13 cells/mm²) compared with the ileum (about 5 cells/mm²). PYY-IR cells were found more frequently in the large intestine (8–18 cells/mm²) than in the small intestine. SP-IR cells were most abundant in the cecum (about 18 cells/mm²). Enteroglucagon-IR cell numbers increased towards the ileum (about 18 cells/mm²), while they were less numerous in the large intestine than in the distal small intestine. Pancreatic glucagon-IR cells could not be detected in the intestine.

In Brunner's glands located in the narrow zone of the

initial duodenum, 9 types of endocrine cells immunoreactive for chromogranin, serotonin, gastrin, somatostatin, BPP, enteroglucagon, PYY, motilin, and neurotensin were also detected. However, the latter 5 types of endocrine cells were very few in number (less than 1 cell/mm²).

DISCUSSION

Immunohistochemical studies regarding the type, regional distribution and relative frequency of gut endocrine cells in the whole length of gastrointestinal tract have not been reported for the common tree shrew (*Tupaia belangeri*). Fourteen types of endocrine cells immunoreactive for chromogranin, serotonin, gastrin, bovine pancreatic polypeptide (BPP), somatostatin, pancreatic glucagon,

enteroglucagon, secretin, cholecystokinin (CCK), gastric inhibitory peptide (GIP), neurotensin, motilin, peptide tyrosine tyrosine (PYY) and substance P (SP) were found in the present study. These were classified into 3 groups according to their distribution. The first group which included chromogranin- and serotonin-immunoreactive (IR) cells was distributed throughout the entire gastrointestinal tract. The second group was distributed mainly in the stomach. This group included endocrine cells immunoreactive for gastrin, BPP, somatostatin, pancreatic glucagon and enteroglucagon. The third group was found mainly in the intestine. This group included endocrine cells immunoreactive for secretin, CCK, GIP, neurotensin, motilin, SP and PYY.

Previous immunohistochemical studies in *T. belangeri* focused only on some gut endocrine cell types. Helmstaedter *et al.* [13, 14] reported on the ultrastructure of neurotensin cells in the gastrointestinal tract and distribution patterns of pancreatic glucagon-, somatostatin- and gastrin-IR cells in the stomach. Forssmann *et al.* [12] described presence of pancreatic polypeptide-IR cells in the stomach and pancreas. In the present study, we found 14 types of gut endocrine cells present in the gastrointestinal tract and described their distribution and relative frequency along the whole length of gastrointestinal mucosa in the common tree shrew. The types, distribution and relative frequency of gut endocrine cells were similar to those of the human [31] and other mammalian species reported so far [1 (mouse deer), 5 (sheep), 8 (fallow deer), 17 (pig), 18 (mink), 22 (cat), 19 (horse), 20 (cattle), 21 (musk shrew), 41 (bat), 42 (honey possum)]. However, some characteristic findings were noted in the present study. These include a huge number of gastrin-IR cells in the pyloric gland region, numerous serotonin-IR cells in the stomach, appreciable number of BPP-IR cells in the transitional region in the stomach and proper gastric gland region, presence of motilin-IR cells in the pyloric gland region and the large intestine as well as the wide distribution of PYY-IR cells along the whole length of gastrointestinal tract.

Endocrine cells immunoreactive for chromogranin were detected in all regions examined. Whilst chromogranin is not a gut hormone, rather it is a Ca-binding protein in the endocrine granules [32], it is used as a general marker for endocrine cells, although it does not detect all types of gut endocrine cells. Similarly in the mouse deer, chromogranin-IR cells were the most numerous endocrine cell type in all regions examined [1]. In the present study, together with serotonin-IR cells, chromogranin-IR cells were the most prominent endocrine cell type in the whole length of gastrointestinal mucosa in terms of distribution and number except for gastrin-IR cells in the pyloric gland region.

Helmstaedter *et al.* [13] examined regional distribution of glucagon, somatostatin and gastrin cells in the stomach of *T. belangeri*. They reported that somatostatin cells were co-localized with glucagon cells in the fundic gland region and with gastrin cells in the pyloric gland region, respectively. They concluded that the release of gastrin and

glucagon may be regulated by somatostatin. We reconfirmed their results in general and agreed with their conclusion. In Fig. 8 of Helmstaedter *et al.* [13], they illustrated a region where there were no endocrine cells. When we compared their results with our present results, we estimated that the "blank region" might be corresponded to our transitional regions. Although glucagon- and gastrin-IR cells were also absent from the "blank region" in the present study, we demonstrated numerous serotonin-IR cells, moderate number of somatostatin-IR cells, appreciable number of BPP-IR cells and a few SP-IR cells in this "blank region". Therefore we concluded that there are no regions of the stomach lacking endocrine cells in the common tree shrew (*T. belangeri*).

In the present study, serotonin-IR cells were the most numerous endocrine cell type in all of the stomach regions except for the huge number of gastrin-IR cells in the pyloric gland region. Serotonin-IR cells were the second most numerous endocrine cell type in the pyloric gland region and were more numerous than somatostatin-IR cells in every region in the stomach. It is well known that serotonin plays as inhibitor in histamine-stimulated gastric acid secretion [15, 24]. It is possible that serotonin-IR cells might play an important role in the peripheral regulation of gastric acid secretion together with other gut hormones, gastrin and somatostatin. Appreciable number of BPP-IR cells in the stomach was found in the present study. However, they might not play important role in the regulation of gastric acid secretion as serotonin, gastrin and somatostatin in this species, because pancreatic polypeptide is a weak stimulator of basal gastric acid secretion [30].

It is well known that motilin-IR cells are critically important endocrine cells of the proximal small intestine [23]. In the present study, however, they were detected in the pyloric gland region as well as in the whole length of the intestine. To date, the presence of motilin-IR cells in the pyloric gland region has been reported in some reptiles [2, 40] and birds [39], but not in mammals. The distribution of motilin-IR cells in the large intestine has been reported only in the musk shrew [21] and lesser mouse deer [1]. Although motilin has a significant stimulation of gastrointestinal and lower esophageal motilities in the dog and in humans [36], the functional significance of the wide distribution of motilin-IR cells in the tree shrew is obscure.

In the present study, we found PYY-IR cells in all segments of the gastrointestinal tract except in the transitional region of the greater curvature. To our knowledge, PYY-IR cells have been found previously only in the distal parts of the intestine in rat [12], pig [3] and humans [10]. PYY has been reported to inhibit gastric emptying, gastric secretion and pancreatic secretion [37], but functional significance of its wide distribution in the tree shrew is unknown.

In conclusion, the regional distribution and relative frequency of gut endocrine cells of *T. belangeri* were clarified in this study. The distribution pattern of gut endocrine cells in the gastrointestinal tract of the common

tree shrew was fundamentally similar to those previously reported for other mammals including humans [31], insectivorous musk shrew [21] and insectivorous bat [41]. The significant findings in the present study are the huge number of gastrin-IR cells in the pyloric region, numerous serotonin-IR cells in the stomach, appreciable number of BPP-IR cells in the transitional regions of the stomach, and wide distribution of motilin- and PYY-IR cells in the gastrointestinal tract.

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