Gut Endocrine Cells in Phyllostomid Bats

An Immunohistochemical Study of Gut Endocrine Cells in Nectarivorous and Frugi-nectarivorous Phyllostomid Bats (Chiroptera: Anoura caudifer and Carollia perspicillata)

Nagatoshi ASHIHARA\(^{1,}\), Valdir A. TADDEI\(^{2,}\), Eiichi HONDO\(^{3,}\), Nobuo KITAMURA\(^{4,}\)
Vitalino D. PAI\(^{5,}\), Valencio J. D. M. CAMPOS\(^{6,}\), Chairun N. CHOLIQ\(^{8,}\) and Junzo YAMADA\(^{9,}\)

1) Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan
2) Department of Morphology, São Paulo State University 'Julio de Mesquita Filho' (UNESP), Botucatu-SP, 18600, Brazil
3) Department of Zoology, São Paulo State University 'Julio de Mesquita Filho' (UNESP), São Jose de Rio Preto-SP, 15100, Brazil
4) Department of Veterinary Anatomy, Bogor Agricultural University, Bogor 16151, Indonesia (1999.6.9 received, 1999.7.19 accepted)

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(Anoura caudifer および Carollia perspicillata)の消化管内分泌細胞の免疫組織化学的研究

芦原 永敏\(^{1,}\), V.A. タデイ\(^{2,}\), 本道 孝一\(^{3,}\), 北村 延夫\(^{4,}\)
V.D. パイ\(^{5,}\), V.J.D.M. カンポス\(^{6,}\), C.N. ホリク\(^{8,}\), 山田 純三\(^{9,}\)

1) 帯広畜産大学畜産学部家畜解剖学教室 〒 080-8555 帯広市稲田町西2
2) サンパウロ州立大学サンジョセドリオプレット校動物学教室 サンジョセドリオプレット、15100、ブラジル
3) サンパウロ州立大学ポツカツ形態学教室 ポツカツ、18600、ブラジル
4) ボゴール農業大学獣医解剖学教室 ボゴール、16151、インドネシア

ABSTRACT. The distribution and relative frequency of gut endocrine cells were studied in nectarivorous bats, Anoura caudifer and frugi-nectarivorous bats, Carollia perspicillata by immunohistochemistry. Five kinds of endocrine cells immunoreactive for either serotonin, somatostatin, gastrin, enteroglucagon or bovine pancreatic polypeptide (BPP), were observed in the stomach. Serotonin-immunoreactive (IR) cells were abundant in the cardiac, fundic and pyloric glands, whereas gastrin-IR cells were very numerous and somatostatin-IR cells were abundant in the pyloric glands. A few enteroglucagon- and BPP-IR cells were found only in the fundic and cardiac glands, respectively. In addition to the five immunoreactive cell types observed in the stomach, endocrine cells immunoreactive for motilin, gastric inhibitory polypeptide (GIP) and neurotensin were detected in the intestine. Serotonin-IR cells were most abundant along the length of the intestine. Serotonin-, somatostatin-, gastrin- and BPP-IR cells also were found in Brunner's glands.

Key Words: gut endocrine cell, digestive tract, immunohistochemistry, Phyllostomid bats, Chiroptera
INTRODUCTION

Bats of the family Phyllostomidae have widely diversified dietary habits. It is of significance to understand how the structural features of the gastrointestinal tract of these bats have adapted to various dietary habits. The gastrointestinal tracts of bats were studied previously by histological, histochemical and ultrastructural means [1, 3, 4, 5, 7, 8, 16, 19, 22]. Recently the distribution and frequency of gut endocrine cells in sanguivorous and insectivorous bats were examined by immunohistochemistry [24, 26]. Nine types of endocrine cells were demonstrated in the gastrointestinal tract of sanguivorous bats and eleven types in insectivorous bats. Ultrastructural and immunohistochemical studies also demonstrated gastrin cells within the pyloric mucosa of some phyllostomid bats [13]. Immunohistochemical studies on gut endocrine cells have yet to be done on nectarivorous and frugivorous bats. The present study examined the distribution and relative frequency of the gut endocrine cells in the frugi-nectarivorous phyllostomid bat, Carollia perspicillata and the nectarivorous phyllostomid bat, Anoura caudifer, by use of immunohistochemical probes.

MATERIALS AND METHODS

Tissues from the nectarivorous bats, Anoura caudifer (2 males and 1 female, weighing 10-11 g) and the frugi-nectarivorous bats, Carollia perspicillata (2 males and 3 females, weighing 13-16 g) were collected near São Jose de Rio Preto, Brasil. The bats were killed by decapitation following etherization. The gastrointestinal tracts were removed and fixed with Bouin's fluid. Following fixation, they were transferred into 70% alcohol, and then separated into the eight regions as shown in Fig. 1.

The tissues were dehydrated in alcohol, cleared in xylene, embedded in paraffin, cut at 5 μm, mounted on gelatin-coated slides, and immunostained by the avidin-biotin-peroxidase complex (ABC) method [6] using a Vectastain ABC kit (Vector Lab. Inc., Burlingame, U.S.A.). Details of the specific antisera used are shown in Table 1. The specificity of each of the immunohistochemical reaction was determined as recommended by Sternberger [18], including replacement of a specific antiserum by the same antiserum preincubated with its corresponding antigen.

After immunohistochemical staining, the sections were lightly counter-stained with Mayer's hematoxylin, dehydrated, cleared in xylene, and mounted. To evaluate the relative frequency of immunoreactive cells, the number of immunoreactive cells per unit area (0.25 mm²) of mucosa was calculated by use of a computerized image analyzer (Spicca, Nippon Avionics, Tokyo, Japan). The frequency of occurrence was shown as a mean + SD (standard deviation) per the unit area. The relative frequency of immunoreactive cells in each region of the both species is shown in Table 2. In this study, 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 [26].

RESULTS

The gastric mucosa of both species of bats (nectarivorous Anoura caudifer and frugi-nectarivorous Carollia perspicillata) contained cardiac, fundic and pyloric glands. The cardiac glands were restricted to a narrow portion of each stomach where it joined the esophagus (Fig. 1). The lumen of the pylorofundic transitional zone was markedly
The majority of gastric mucosa was comprised of fundic (oxyntic) glands in the both species.

The length of the intestine was about 16 cm in *Carollia* and 11 cm in *Anoura*. Their intestines were similar in macroscopic appearance. Because both species lack a caecum, there was no obvious subdivision between small and large intestines. Intestinal tissues were taken for examination from five different regions (Fig. 1). Brunner’s glands were restricted in distribution to the proximal duodenum at the pyloroduodenal junction.

Eight types of endocrine cells immunoreactive for either serotonin, somatostatin, gastrin, gastric inhibitory polypeptide (GIP), motilin, neurotensin, enteroglucagon, or bovine pancreatic polypeptide (BPP) were identified in the gastrointestinal mucosa of both species (Table 2). Neither cholecystokinin-nor secretin-immunoreactive (IR) endocrine cells were observed in either species. Serotonin- and somatostatin-IR cells were found in all regions examined, but other types of immunoreactive cells showed some restricted distributions. The distribution and frequency of the eight types of endocrine

![Diagram](attachment:image.png)

**Fig. 1** Schematic drawing of the digestive tract illustrating regions sampled from *Anoura caudifer* and *Carollia perspicillata*.

1. Cardiac glands
2. Fundic glands
3. Pyloric glands
4. Brunner’s glands
5. Duodenum (Initial region)
6. Middle region of proximal half of intestine
7. Middle region of whole length of intestine
8. Middle region of distal half of intestine
9. Terminal region of intestine

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**Table 1** Antisera used

<table>
<thead>
<tr>
<th>Antisera</th>
<th>Code or Lot No.</th>
<th>Specificity</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin*</td>
<td>16302</td>
<td>-</td>
<td>1:10,000</td>
</tr>
<tr>
<td>Synthetic human cyclic somatostatin*</td>
<td>-</td>
<td>-</td>
<td>1:3,000</td>
</tr>
<tr>
<td>Bovine pancreatic polypeptide*</td>
<td>615-R-110-146-17</td>
<td>Cross-reacts with human pancreatic polypeptide</td>
<td>1:10,000</td>
</tr>
<tr>
<td>Natural porcine glucagon*</td>
<td>RPN.1602</td>
<td>Reacts with pancreatic and intestinal glucagon</td>
<td>1:1,000</td>
</tr>
<tr>
<td>Natural porcine cholecystokinin*</td>
<td>RPN.1742</td>
<td>Reacts with CCK 33/CCK 39, no cross-reaction with gastrin</td>
<td>1:1,000</td>
</tr>
<tr>
<td>Gastric inhibitory polypeptide*</td>
<td>G/R-34-III D</td>
<td>No cross-reaction with glucagon</td>
<td>1:10,000</td>
</tr>
<tr>
<td>Synthetic porcine motilin*</td>
<td>R-1104</td>
<td>Reacts against entire molecules</td>
<td>1:1,000</td>
</tr>
<tr>
<td>Synthetic human gastrin*</td>
<td>GP-1304</td>
<td>No cross-reaction with cholecystokinin</td>
<td>1:10,000</td>
</tr>
<tr>
<td>Synthetic porcine secretin*</td>
<td>R-801</td>
<td>Reacts with the C- and N-terminals</td>
<td>1:1,000</td>
</tr>
<tr>
<td>Synthetic bovine neurotensin*</td>
<td>R-3501</td>
<td>-</td>
<td>1:1,000</td>
</tr>
</tbody>
</table>

*: All antisera were raised in rabbits except those against gastrin which were raised in guinea pigs. 
, , and : These antisera were purchased from Immunonuclear Corp., Stillwater, U.S.A.; Amersham International plc., Amersham, U.K.; Guildhay, Surrey, U.K., respectively. 
, , and : These antisera were kindly donated by Dr. S. Ito, Niigata, Japan; Dr. R.E. Chance, Indianapolis, U.S.A.; Dr. N. Yanaihara, Shizuoka, Japan, respectively.
Table 2 Distribution and relative frequency of gut endocrine cells of Anoura cristata and Carolia perspicillata

<table>
<thead>
<tr>
<th>Endocrine cells</th>
<th>Stomach</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Serotonin</td>
<td>+/++</td>
<td>+/++</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>f/f</td>
<td>+/+</td>
</tr>
<tr>
<td>Gastrin</td>
<td>f/+</td>
<td>f/-</td>
</tr>
<tr>
<td>Motilin</td>
<td>--/--</td>
<td>--/--</td>
</tr>
<tr>
<td>GIP</td>
<td>--/--</td>
<td>--/--</td>
</tr>
<tr>
<td>Neurotensin</td>
<td>--/--</td>
<td>--/--</td>
</tr>
<tr>
<td>Enteroglucagon</td>
<td>--/+</td>
<td>--/+</td>
</tr>
<tr>
<td>BPP</td>
<td>--/+</td>
<td>+/+</td>
</tr>
<tr>
<td>Secretin</td>
<td>--/-</td>
<td>--/-</td>
</tr>
</tbody>
</table>

*Anoura/Carolla, - : not detected, f : few (not detected in every animal), 0<+≤1 cell/0.25 mm², 1<+≤5 cells/0.25 mm², 5<+≤25 cells, 25 cells<+++, 1: cardiac glands, 2: fundic glands, 3: pyloric glands, 4: Brunner's glands, 5: duodenum (initial region of the intestine), 6: middle region of proximal half of intestine, 7: middle region of whole length, 8: middle region of distal half, 9: terminal region, GIP: gastric inhibitory polypeptide, BPP: bovine pancreatic polypeptide, CCK: cholecystokinin.

Cells identified are summarized in Table 2.

In the stomach, five types of endocrine cells immunoreactive for either serotonin, somatostatin, gastrin, enteroglucagon or BPP were found (Table 2 and Figs. 2-8). The majority were round or oval in shape (Figs. 2-8), however, a few pyramidal shaped cells also were present. Enteroglucagon- and BPP-IR cells were primarily of the round and oval variety (Figs. 4, 5). Although they were scattered throughout the cardiac and fundic glands, they were confined primarily in the basal portions of the glands. A small number of them were found within the surface epithelium and in the neck region of the pyloric glands. Serotonin-IR cells were found throughout the gastric mucosa (8.38 ± 3.77 cells/0.25 mm² in the cardiac glands; Fig. 2, 11.55 ± 6.96 in the fundic glands, 18.45 ± 12.88 in the pyloric glands; Fig. 6) of Carolia. Few serotonin-IR cells were in the cardiac and fundic glands in Anoura, but more numerous in the pyloric glands (21.25 ± 3.31). Somatostatin-IR cells were also abundant in the pyloric glands (10.42 ± 4.53 in Carolia; Fig. 7, 20.20 ± 7.49 in Anoura), but were few in the cardiac and fundic glands of both species. Gastrin-IR cells were detected in all gastric glands of both species except for the fundic glands of Carolia. They were very numerous (103.90 ± 39.18 in Carolia; Fig. 8, 62.40 ± 12.58 in Anoura) in the pyloric glands, but were few or not detected in each section of the cardiac and fundic glands of Anoura and were a few in the cardiac glands of Carolia (Fig. 3). Enteroglucagon-IR cells were found in the fundic glands (Fig. 4). A very small number of BPP-IR cells were found in the fundic and pyloric glands in both species (Fig. 5). The immunoreactive endocrine cells in the cardiac and fundic glands were primarily of the closed type that had no contact with glandular or gastric lumen. On the other hand, those of the pyloric glands frequently exhibited an apical cytoplasmic process in contact with the lumen and were judged to be the open type.

In the intestine, eight types of immunoreactive endocrine cells were recognized in both species (Table 2 and Figs. 9-15). Here endocrine cells were slender and elongated or triangular and were in contact with the intestinal or glandular lumen (Figs. 9-12). Motilin-, GIP- and neurotensin-IR cells were obser-
Gut Endocrine Cells in Phyllostomid Bats

Figs. 2–8. Endocrine cells in the stomach. ×400.

Fig. 2. Four serotonin-IR cells in the cardiac glands of *Carollia*. Stratified squamous epithelium of the esophagus is shown at the right of the figure.

Fig. 3. Three gastrin-immunoreactive (IR) cells in the cardiac glands of *Carollia*. This is an adjacent section of Fig. 2.

Fig. 4. Two enteroglucagon-IR cells in the fundic glands of *Anoura* are round in shape.

Fig. 5. Two round BPP-IR cells in the fundic glands of *Anoura*. These cells are located in the glandular bases.

Figs. 6–8. Figs. 6, 7 and 8 are serial section of the pyloric glands of *Carollia*. Some of serotonin (Fig. 6)-, somatostatin (Fig. 7)- and gastrin (Fig. 8)-IR cells are found in the glandular bases. ×400.

...serotonin-IR cells were found most frequently in the intestinal villi, whereas enteroglucagon-IR cells were found most frequently in the intestinal glands. Serotonin-IR cells were abundant while somatostatin-IR cells were few along the entire length of the intestine in both species. A few gastrin-IR cells were also detected throughout the intestine in both species (Fig. 10) and decreased in number distally. Motilin- and GIP-IR cells were observed primarily in the proximal intestine (Fig. 9), decreased distally and were few in number in the terminal region of intestine of both species. Neurotensin-IR cells were also observed in the distal intestine (with the exception for the terminal region) of both species (Fig. 11). They decreased gradually in number proximally in both species but were not found in the duodenum of *Anoura*. Enteroglucagon-IR cells were found throughout the intestine (Fig. 12), except in the duodenum of *Anoura*. They were relatively abundant in the distal intestine (about 6 cells per unit) of *Anoura*, and appeared to increase in number distally along the intestine of both species. A very small number of BPP-IR cells were observed mainly in middle region of the intestine.

Serotonin-, somatostatin-, gastrin- and BPP-IR cells were observed among the exocrine cells of Brunner's glands of both species (Figs. 13–15). Their relative frequencies appeared higher in *Anoura* than in *Carollia* (Table 2). The endocrine cells...
showing open type were not found in Brunner's glands.

**DISCUSSION**

The present study documents the distribution and relative frequency of 8 kinds of endocrine cells in the gastrointestinal tract of two species of bats belonging to the family Phyllostomidae. When the frugi-nectarivorous bats, *Carollia perspicillata*, and nectarivorous bats, *Anoura caudifer* were compared to sanguivorous bats, *Desmodus rotundus* [24] and two species of insectivorous bats, *Pipistrellus abramus* (*Pipistrellus javanicus*) and *Plecotus auritus sacrinontis* [26], some differences were found with regard to the appearance, regional distribution and relative frequency of the gut endocrine cells.

The most remarkable difference concerns secretin- and cholecystokinin (CCK)-immunoreactive (IR) cells. Although secretin- and CCK-IR cells were described in the intestine of the sanguivorous (Phyllostomidae, Desmodontidae) and insectivorous (Vespertilionidae) bats [24, 26], they were absent in the frugi-nectarivorous and nectarivorous bats examined in this study. The presence of these two types of endocrine cells in the proximal small intestine is well established in higher mammal (eutherian) [20]. In the lower mammal (prototherian and metatherian), their appearance in the duodenum is inconsistent. Secretin-IR cells were not observed in either the echidna or platypus (prototherian), while CCK-IR cells were found in both species but were very few [23, 25]. In marsupial (metatherian), secretin-IR cells were found to be very few in the short-nosed bandicoot, commonly observed in koala and Virginia opossum, but were not observed in the tiger cat, great gray kangaroo, Parma wallaby, common brush-tail possum, ring-tailed possum, common wombat and honey possum [21]. Cholecystokinin-IR cells were found regularly in the Virginia opossum but were observed infrequently in koala, common brush-tail possum, common wombat, tiger cat and honey possum [21]. They were not observed in other marsupial species examined [11, 21, 27]. The inconsistent appearance of secretin- and

Figs. 9~11. Endocrine cells in the intestine. Four kinds of IR cells demonstrated in the intestinal mucosa. These endocrine cells are open type having a luminal contact with an apical cytoplasmic process. ×400.

**Fig. 9.** A motilin-IR cell in the duodenum of *Carollia*.

**Fig. 10.** A gastrin-IR cell in the mid-region of the intestine of *Anoura*.

**Fig. 11.** A neurotensin-IR cell from the mid-region of the distal half of the intestine of *Carollia*.

**Fig. 13.** An enteroglucagon-IR cell in the terminal region of intestine of *Carollia*.

**Figs. 13~15.** Three types of immunoreactive cells in the three adjacent sections from Brunner's glands from *Anoura*. Somatostatin (Fig. 12)-, gastrin (Fig. 13)- and serotonin (Fig. 14)-IR cells are shown. ×400.
CCK-IR cells in the duodenum of these species as well as bats may contribute to the better understanding of the evolution of these hormones. The absence of immunoreactivities by antisera against porcine secretin and CCK may be due to difference in their molecular forms in the two species of bats or may reflect a real absence. Because secretin- and CCK-IR cells could be demonstrated in other species of bats, one would predict that the lack of immunoreactivity does represent an absence of these two cell types in frugi-nectarivorous and nectarivorous bats.

Another remarkable difference found concerned the distribution of gastrin-IR cells in the stomach. Gastrin-IR cells were detected in all the gastric glands of nectarivorous bats and in cardiac and pyloric glands of the frugi-nectarivorous bats. In contrast, they were restricted in distribution to the pyloric glands in two species of insectivorous and sanguivorous bats [24, 26]. A functional meaning of the presence of a few gastrin-IR cells in the fundic and cardiac glands is not yet clear.

The distribution and relative frequency of somatostatin- and serotonin-IR cells in nectarivorous and frugi-nectarivorous bats were similar to that of the sanguivorous and insectivorous bats [24, 26]. In the stomach, however, somatostatin-IR cells were less abundant in the fundic glands of nectarivorous and frugi-nectarivorous bats than in sanguivorous and insectivorous bats. Serotonin-IR cells were not abundant in the fundic glands of nectarivorous bats [24, 26]. Motilin-IR cells were lacking in the gastrointestinal tract of the sanguivorous bats [24], whereas their distribution and relative frequency in nectarivorous and frugi-nectarivorous bats were similar to those of insectivorous bats [26]. The distribution and relative frequency of GIP- and neuropeptide-IR cells in the nectarivorous and frugi-nectarivorous bats were similar to that of insectivorous and sanguivorous bats extending from the duodenum to the middle region of the distal half of intestine. The distribution of enteroglucagon-IR cells in nectarivorous and frugi-nectarivorous bats was similar to that of the insectivorous bats [26] and the distribution of glicentin-IR cells in sanguivorous bats [24]. Enteroglucagon-IR cells in insectivorous bats were of moderate number [26], but they were few in nectarivorous and frugi-nectarivorous bats. The distribution of BPP-IR cells was different in each species of bats examined. In the stomach, BPP-IR cells were found in the fundic and pyloric glands of insectivorous bats [26] but not found in the sanguivorous bats [24]. In the intestine, BPP-IR cells were observed from the middle region of the proximal half to the middle region of the distal half of the intestine in nectarivorous and frugi-nectarivorous bats. They were detected along the length of the intestine of insectivorous bats [26], but they were not seen in sanguivorous bats [24].

The sanguivorous bats (Desmodus), insectivorous bats (Pipistrellus and Plecotus) and the frugi-nectarivorous and nectarivorous bats (Carollia and Anoura) are classified into 3 different families, Desmodontidae, Vespertilionidae and Phyllostomatidae, respectively. It is unknown whether the differences in the distribution and relative frequency of gut endocrine cells described here are due to differences in their dietary habits. In studies of other nectarivorous animals, the New Holland honeyeater (birds) [17] and the honey possum (marsupials) [27], a decreasing trend in types and frequency of gut endocrine cells was suggested. Such a tendency, although not as obvious, was recognized in this study. The nectarivorous and frugivorous bats were ideal animals in which to study the relationship between the morphology of the gastrointestinal tract and the dietary habits of closely related animals. There have been some studies that have examined efficiency of food utilization, digestive enzymes and food transit time in frugivorous bats [2, 9, 10, 12].
If nectarivorous and frugivorous bats were compared to other bats, which differ in dietary habits, differences in the distribution and relative frequency of gut endocrine cells would be predicted. Immunohistochemical and ultrastructural studies on the gastrointestinal tract of bats including those in the present study, revealed apparent interspecies differences among them [13, 19, 24, 26]. Although such interspecies differences are difficult to interpret, the present results suggest a certain correlation between the distribution of endocrine cells regulating gastrointestinal function and dietary habits. The relationships between gut endocrine cells and diet of other species of phyllostomid bats should be investigated further by use of immunohistochemical methods before precise relationships can be fully appreciated.

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Gut Endocrine Cells in Phyllostomid Bats


