IMMUNOHISTOCHEMICAL STUDY ON THE DISTRIBUTION AND FREQUENCY OF GUT ENDOCRINE CELLS IN THE STOMACH OF INSECTIVOROUS VESPERTILIONID BAT, *SCOTOPHILUS KUHLII*

KAJIAN IMUNOHISTOKIMIA TERHADAP DISTRIBUSI DAN FREKUENSI SEL-SEL ENDOKRIN PADA LAMBUNG KELELAWAR PEMAKAN SERANGGA, *SCOTOPHILUS KUHLII*

Chairun Nisa' [1], Srihadi Agungpriyono[1], Koeswinarning Sigit [1], Agustinus Suyanto[3], Nobuo Kitamura[2] and Junzo Yamada[2]

[1]Department of Anatomy, Faculty of Veterinary Medicine,Bogor Agricultural University, Kampus IPB Darmaga, Jl. Agatis , Bogor, 16680, INDONESIA, e-mail : chnisa@yahoo.com, [2]Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho Obihiro 080, JAPAN, [3]Research and Development Centre of Biology, Indonesian Institute of Science, Jl. Raya Cibinong Bogor, INDONESIA

ABSTRACT

Media Veteriner. 2000.7(4):1-5.

The distribution and frequency of gut endocrine cells in the stomach of insectivorous vespertilionid bat *(Scotophilus kuhlii)* were studied by specific immunohistochemical technique, the avidin-biotin peroxidase complex (ABC) method according to Hsu *et al.* (1981), using seven kinds of antisera: chromogranin, serotonin, somatostatin, gastrin, bovine pancreatic polypeptide (BPP), substance-P and glucagon. Cells immunoreactive for chromogranin, serotonin and BPP were found in all glandular portion of the stomach, the cardiac, proper gastric and pyloric glands regions. The number of chromogranin immunoreactive cells varied from a few to numerous, serotonin immunoreactive cells were from a few to moderate while those of BPP immunoreactive cells were rare to moderate. Moderate to numerous numbers of cells immunoreactive to gastrin and substance-P were confined only to the pyloric gland region, while a few to moderate number of cells immunoreactive to somatostatin were distributed in the proper gastric and pyloric glands regions. Moderate number of glucagon immunoreactive cells were confined only in the proper gastric gland region. The endocrine cells were open and closed types with or without basal cytoplasmic process and variation in their shapes: round, oval, pyramidal and spindle shapes. The distribution pattern of endocrine cells in *S. kuhlii* was almost similar to those of other mammals so far reported. However, special attention should be noted on the absence of endocrine cells immunoreactive to somatostatin in the cardiac gland region and the presence of BPP immunoreactive cells in all portion of the stomach glands and endocrine cells immunoreactive to substance P in the pyloric gland region of *S. kuhlii.* These findings might suggest to be related with the digestive function of the stomach of this animal species.

Key words: stomach, endocrine cells, immunoreactive, antisera, insectivorous bat

ABSTRAK

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Morfologi, distribusi dan frekuensi relatif sel-sel endokrin pada lambung kelelawar pemakan serangga *(Scotophilus kuhlii)* telah dipelajari dengan menggunakan teknik imunohistokimia spesifik avidin-biotin peroxidase complex (ABC method) menurut Hsu *et al.* (1981) memakai tujuh macam antibodi terhadap chromogranin, serotonin, somatostatin, gastrin, bovine pancreatic polypeptide (BPP), substance P dan glucagon. Sel-sel imunoreaktif terhadap chromogranin, serotonin dan BPP ditemukan pada semua daerah kelenjar lambung kardia, fundus dan pilorus. Sel-sel yang imunoreaktif chromogranin ditemukan sedikit sampai banyak, serotonin sedikit sampai sedang, sedangkan BPP jarang sampai sedang. Sel-sel imunoreaktif terhadap gastrin dan substance P hanya ditemukan di daerah pilorus dalam jumlah sedang sampai banyak. Sel-sel imunoreaktif somatostatin ditemukan dalam jumlah sedikit di daerah fundus dan sedang di pilorus, sedangkan sel-sel imunoreaktif glucagon dalam jumlah sedikit sampai sedang di temukan terbatas hanya pada daerah fundus. Morfologi sel-sel endokrin yang teramati terdiri atas tipe terbuka dan tertutup dengan atau tanpa penjuluran sitoplasma serta bentuk bervariasi seperti bulat, oval, segitiga dan *spindle.* Gambaran penyebaran sel-sel endokrin ini mirip dengan gambaran yang dilaporkan pada hewan mamalia lainnya. Tetapi ada beberapa hasil menarik yang dapat dicatat dari penelitian ini, yaitu tidak ditemukannya sel-sel imunoreaktif somatostatin di daerah kardia, ditemukannya sel-sel imunoreaktif BPP pada semua daerah kelenjar lambung serta ditemukannya sel-sel imunoreaktif substance P pada daerah pilorus. Hasil ini diduga berkaitan dengan fungsi lambung dalam proses pencernaan hewan ini.

Kata-kata kunci: lambung, sel-sel endokrin, imunoreaktif, antisera, kelelawar pemakan serangga

INTRODUCTION

The vespertilionids bats are common insectivorous bat that classified in the subordo Microchiroptera. Most vespertilionids acquire their food in the air, so they prey flying insect (Hall and Woodside, 1989). The Asian lesser yellow house bat, *Scotophilus kuhlii,* is a relative big vespertilionid bat. The body weight is about 15-22 g (Lekagul and Mc Neely, 1977), with the length of fore arm is about 45-59 mm. In Indonesia this species distributes widely, except Irian island and some small islands around (Corbet and Hill, 1992).

The morphology of the digestive tract of *S. kuhlii* that has been observed in our previous study (Nisa', 1997) is characterized by simple stomach and short intestine without caecum. The stomach is about 1.27 cm length and 0.7 cm width, globular in shape with a short fundic caecum. The esophageal entrance is adjacent to the duodenal orifice. The glandular areas of the stomach consist of cardiac, proper gastric and pyloric glands, respectively. The cardiac gland region is very narrow, with 2-5 glands scattered at the junction between stratified squamous epithelium of the esophagus and the proper gastric gland. The proper gastric glands are distributed widely, while the pyloric glands are confined to the area adjacent to the pyloric-duodenal junction. Transitional glandular areas between cardiac to proper gastric glands and proper gastric to pyloric glands are also found in the stomach of *S. kuhlii*. Despite many data for stomach morphology, studies on the gut endocrine cells in the stomach has not yet been reported in *S. kuhlii* species. Studies of gut endocrine cells of insectivorous bats have been reported only in *Pipistrelus abramus* and *Plecotus auritus sacrimontis* (Yamada *et al.*, 1988).

This study aims to reveal the types and distribution of gut endocrine cells in the stomach of *S. kuhlii*, using seven specific antisera to gastrointestinal hormones. The obtained data will provide a better understanding on the function of the stomach in this animal species.

MATERIALS AND METHODS

Five animals (*S. kuhlii*) of both sexes (body weight 16.3-28.9 g) were used in this study. The animals were caught in the Bogor Botanical Garden under permission. The animals were sacrificed under anesthesia by ether inhalation. Incision was made along the median line (*Linea alba*) and immediately the gastrointestinal tract was injected with the Bouin fluid in the several points along the digestive tract to facilitate a better penetration of fixative solution. The gastrointestinal tract was removed from the body, washed with 0.1 M phosphate buffered saline (PBS, pH 7.4) and fixed immediately in Bouin fluid for 24 hours and then transferred into 70% alcohol. The whole stomach was taken and cut along the median. Each half of the stomach was processed routinely for embedding in paraffin. Sections of 5 µm thick were cut serially. After deparaffinized sections were treated with 0.3% H_2O_2 in methanol for 10 minutes to block any endogenous peroxidase and then were incubated with normal rabbit (only for Substance P) or goat (for another antisera) serum at room temperature for 30 minutes. The sections were then stained immunohistochemically using ABC method (Hsu *et al.*, 1981) to demonstrate specific gut endocrine cells. Details of specific antisera used are shown in Table 1. The sections were incubated with the specific antisera as the first layer overnight at 4°C. As the second layer biotinylated anti rabbit IgG serum raised in goat (Vector, Burlingame, Calif., USA, code BA-1000 for all antisera except those to gastrin and substance P) or biotinylated anti guinea pig IgG serum raised in goat (Vector, code BA-7000 for gastrin) or biotinylated anti rat IgG serum raised in rabbit (Vector, code BA-400 for substance P) were used at 1 : 200 for 30 minutes at room temperature. Vectastain ABC kit (Vector, code PK-6100) was used as the third layer at 1 : 2 for 30 minutes at room temperature. 3-3'-diaminobenzidine-hydrochloride (0.02% w/v Tris-HCl buffer; Dojindo, Kumamoto, Japan, code 349-00903) was used to visualize the immunoreactions.

The specificity of each immunoreaction was checked by replacement of specific antiserum with normal serum or phosphate buffer saline.

The distribution and relative frequency of each endocrine cell immunoreactive for each antisera in each region was graded subjectively into five groups according to its occurrence in the visual field of light microscopy.

RESULTS

In the present study seven types of endocrine cells immunoreactive to chromogranin, serotonin, somatostatin, gastrin, BPP, substance P and glucagon were identified in the stomach glands of *S. kuhlii* (Figure 1). The distribution and frequency of each endocrine cell in the stomach glands are summarized in Table 2.

In the cardiac gland region, a few cells immunoreactive to chromogranin and serotonin and rare cells immunoreactive to BPP were found. The cells were open and closed types with oval and pyramidal shapes and were mainly located in the basal part of the glands. Open type cells had luminal contact, while closed type had not.

In the proper gastric glands region, moderate to numerous numbers endocrine cells immunoreactive to chromogranin, serotonin and glucagon. Beside that a few endocrine cells immunoreactive to somatostatin and BPP were also detected. Most of these cells were located in the base of the glands, less number scattered in the neck and rare number were found in the surface epithelium of the proper gastric glands region. The endocrine cells in the proper gastric were mainly closed type with or without basal cytoplasmic process and were round and oval in shapes. Several were open type with pyramidal and spindle shapes. In the pyloric glands region, cells immunoreactive to all types of antisera used, except glucagon were detected. The cells immunoreactive to chromogranin and gastrin were the most numerous, while cells immunoreactive to serotonin, somatostatin, BPP and substance P cells were moderate. All of them were open type endocrine cells, showing pyramidal and spindle in shape.

Table 2. The distribution and relative frequency of gut endocrine cells in the stomach of *S. kuhlii*

Antisera	Cardiac Gland	Fundic Gland			Pyloric Gland
		Basal	Middle	Apical	
Chromogranin	+	+++	+	±	+++
Serotonin	+	++	+	–	++
Somatostatin	–	+	±/+	–	++
Gastrin	–	–	–	–	+++
BPP	±	+	+	–	++
Substance P	–	–	–	–	++
Glucagon	–	++	+	–	–

– absent, ± rare (not detected in every animal), + few, ++ moderate, +++ numerous, BPP = bovine pancreatic polypeptide.

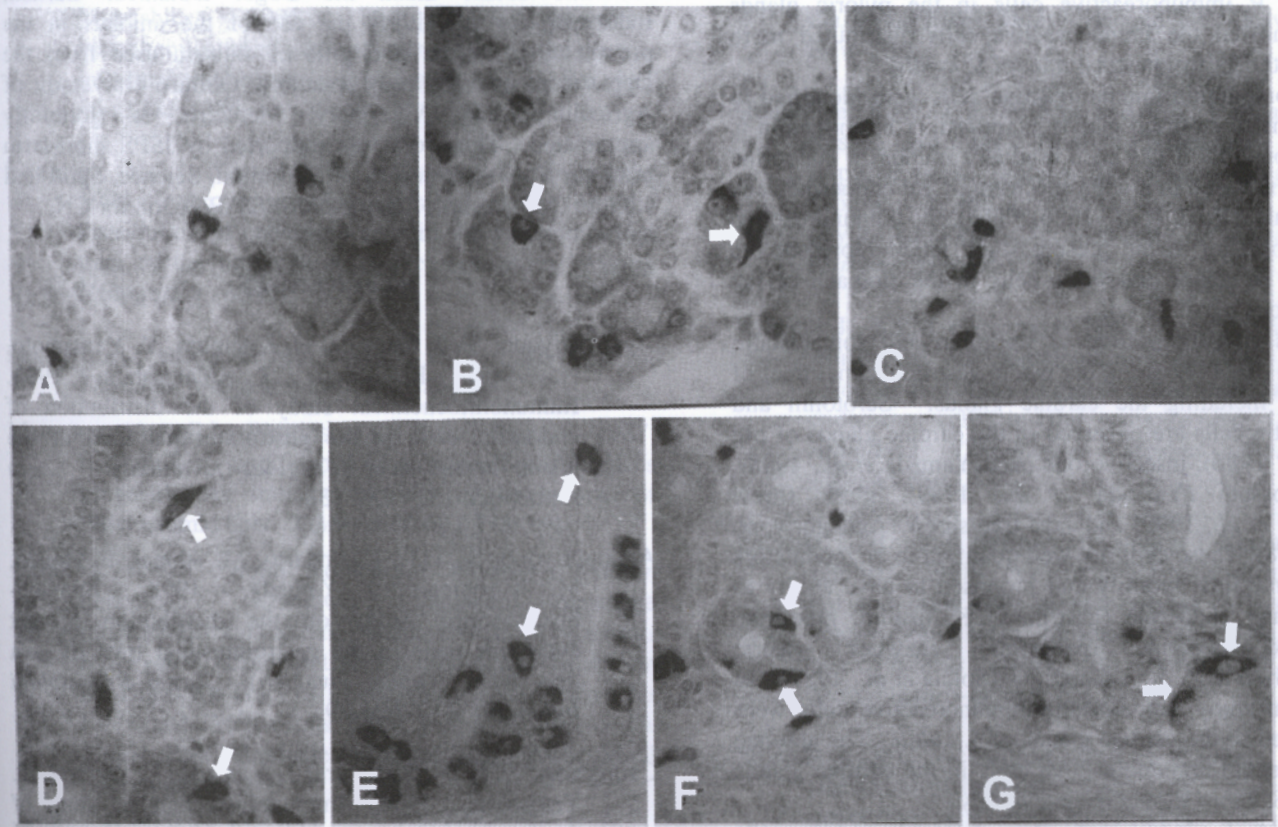

Figure 1. Photomicrograph of gut endocrine cells in the stomach mucosa of *S. kuhlii,* show cells immunoreactive (white arrows) to chromogranin (A), serotonin (B) and glucagon (C) in the proper gastric glands region, and somatostatin (D), gastrin (E), BPP (F) and substance-P (G) in the pyloric glands region. A, B, D, E, G : x 250; C, F : x 165.

DISCUSSION

Seven types of gut endocrine cells immunoreactive to chromogranin, serotonin, somatostatin, gastrin, BPP, substance P and glucagon were identified in the stomach of an insectivorous bat, *Scotophilus kuhlii* which is classified in the family Vespertilionidae. So far, immunohistochemical studies concerning the kinds, distribution and relative frequency of gut endocrine cells in bats have been reported only for the common vampire bat, *Desmodus rotundus* (Yamada *et al.*, 1984), the pyloric G-cells in four phyllostomid genera (*Erophylla, Artibeus, Ariteus and Glossophaga*) (Mennone *et al.*, 1986) and the previous study on the same family of insectivorous bat but different species, *Pipistrelus abramus* and *Plecotus auritus sacrimontis* (Yamada *et al.*, 1988). Although the types, distribution and frequency of gut endocrine cells in the stomach of *S. kuhlii* were similar fundamentally with those of insectivorous bats and with those reported in other mammalian species, some characteristic findings were noted. These included the absence of endocrine cells immunoreactive to somatostatin in the cardiac glands, the presence of endocrine cells immunoreactive to BPP in all portions of the stomach glands and the presence of endocrine cells immunoreactive to substance P in the pyloric glands.

The most interesting finding in the present study is the presence of substance P- immunoreactive cells in the pyloric gland region. At serial section seemed that substance P immunoreactive cells were independent endocrine cells and no co-localization with gastrin and somatostatin immunoreactive cells (Fig.1). The presence of substance P-immunoreactive cells in the pyloric glands region has not been reported yet in the previous studies, included those in the insectivorous bats (Yamada *et al.*, 1988). Substance P-immunoreactive cells are recognized mainly in the small intestine of cattle (Kitamura *et al.,* 1985), sheep (Calingasan *et al.,* 1984, Ceccarelli *et al.,* 1991) and Java tree shrew (Agungpriyono, 1998) and in the large intestine of the lesser mouse deer (Agungpriyono *et al.,* 1994). The presence of substance P immunoreactive cells in the pyloric glands region, showed the immunorective cells distributed more widely in the gastrointestinal tract of *S. kuhlii.*

Chromogranin immunoreactive cells were identified in all portions of stomach glands examined. Chromogranin is not hormone, but it has been used as a marker of endocrine cells as it is found in a wide spectrum of gut endocrine cells in mammals (Rindi *et al.,* 1986). The cells immunoreactive to chromogranin in the present study might represent the frequency of major endocrine cells in the stomach of the insectivorous bat *S. kuhlii.*

Cells immunoreactive to serotonin and somatostatin have been observed along the whole gastrointestinal tract of mammals including the cardiac glands. Serotonin and somatostatin cells are observed in the cardiac glands of pig (Ito *et al.,* 1986), insectivorous bats (Yamada *et al.,* 1988), honey possum (Yamada *et al.,* 1989), musk shrew (Kitamura *et al.,* 1990) and Java tree shrew (Agungpriyono *et al.,* 1998). These cells are well qualified to be modulators of gastrointestinal motility and proposed to have ability to inhibit many endocrine (gastrin, CCK, insulin, glucagon, etc.) and exocrine (pancreatic enzymes, gastric hydrochloride acid, etc.) secretions (Solcia *et al.,* 1987). In the present study, somatostatin-immunoreactive cells were not detected in the cardiac glands of *S. kuhlii.* The absence of somatostatin-immunoreactive cells in the cardiac glands region of *S. kuhlii* should be noteworthy.

In the *S. kuhlii,* we also detected a few cells immunoreactive to BPP in the cardiac glands. The presence of BPP-immunoreactive cells in the all glandular regions of the stomach of *S. kuhlii* is similar with the result of Yamada *et al.,* (1988) in *P. auritus sacrimontis* and Agungpriyono *et al.* (1998) in *T. javanica.* Interspecies difference is noted between the insectivorous bats. BPP immunoreactive cells were detected in all glandular regions of the stomach of *S. kuhlii* and *P. auritus sacrimontis,* while in the stomach of *P. abramus* the cells were found only in the proper gastric glands.

Glucagon-immunoreactive cells detected confined to the proper gastric glands. This finding is similar with those of previous studies reported in the insectivorous bats (Yamada *et al.*, 1988) and in the musk shrew (Kitamura *et al.,* 1990). The glucagon cells have been found in the proper gastric glands of all genera of bats examined thus far by Phillips *et al.* (1984) and Studholme *et al.* (1986), regardless of their dietary habits. Moreover, in the present study we observed the endocrine cells were largely concentrated in the pyloric glands region. The concentrate distribution of endocrine cells at the terminal portions of digestive segments, i.e. the pyloric glands region, ileum and rectum, have been reported in other mammals (Calingasan *et al.,* 1984, Kitamura *et al.*, 1982, Kitamura *et al.*, 1990). Kitamura *et al.* (1982) speculated that these endocrine cells may be involved in a feedback regulatory mechanism of the secretory and motile functions of the segment.

Acknowledgement

The author thank the Bogor Botanical Garden, Indonesia, for permitting the use of the animals in the present study. The kind gifts of antisera used from the Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine is gratefully appreciated. This work was supported by JSPS short-term program from the Ministry of Education, Science, Sport and Culture (Monbusho), Japan No. JSPS/AP/96368 for CN.

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Table 1. Antisera used to study gut endocrine cells in the insectivorous bat, *S. kuhlii*

Antiserum target	Code	Specificity	Working dilution	Source
Chromogranin	8808014	-	1:10,000	Incstar, Stillwater, MN, USA
Serotonin	Lot 16302	-	1:150,000	Immuno Nuclear Corp., Stillwater
Somatostatin	-	-	1:10,000	S. Ito, Niigata, Japan
Gastrin	GP-1304	No cross-reaction with CCK-8	1:5,000	N. Yanaihara, Shizouka, Japan
BPP	Lot 615-R-110-146-17	Cross-reacts with human pancreatic polypeptide	1:10,000	R.E. Chance, Indianapolis, IN., USA
Substance-P	MAS035b	-	1:1,000	Sera-Lab, Crawley Down, UK
Glucagon	RPN. 1602	Completely cross-reacts with pancreatic and intestinal glucagon	1:5,000	Amersham International plc., Amersham, UK

BPP = bovine pancreatic polypeptide