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Research Article
Gut Endocrine Cells in the Stomach of Sunda Porcupines (*Hystrix javanica*)

[1]Teguh Budipitojo, [2]Yuda Heru Fibrianto and [3]Guntari Titik Mulyani

[1]Department of Anatomy,
[2]Department of Physiology,
[3]Department of Internal Medicine, Faculty of Veterinary Medicine, Gadjah Mada University, 55281 Yogyakarta, Indonesia

Abstract

Background and Objective: The regional distribution and relative frequency of gastrointestinal endocrine cells has been found to vary with the animal species and feeding habits. This study aimed to analyze the existence and distribution of immunoreactive endocrine cells to chromogranin, ghrelin, somatostatin, serotonin and gastrin in the gastric mucosa of sunda porcupine (*Hystrix javanica*) using immunohistochemistry. **Materials and Methods:** Five adult sunda porcupines were used without sexual distinction. The presences of endocrine cells were visualized by ABC immunohistochemical methods using five types of polyclonal antisera, specific for chromogranin, ghrelin, somatostatin, serotonin and gastrin. **Results:** In the stomach of sunda porcupine, endocrine cells immunoreactive for all of these markers were observed. The chromogranin-immunoreactive cells were found with greatest frequency, in all stomach regions. In all regions of the stomach, endocrine cells secreting ghrelin were also detected in weaker frequency compare with chromogranin. The somatostatin immunoreactive cells were detected rare in the gastric and fundic regions, serotonin cells were found rare in fundic but few in pyloric regions, while gastrin cells were detected abundance in pyloric region of stomach of the sunda porcupines. Gastrin positive cell was also detected in the duodenal part of sunda porcupine. The chromogranin-positive cells were the most numerous, being more prevalent in the stomach glands. The other endocrine cells were identified in smaller numbers, some of them located in all regions or in specific regions. The finding of these cell types in the mucosal stomach confirms their preferential location in the final portions of the principal regions of the stomach and suggests control by feedback of its functions. **Conclusion:** In conclusion, the present study show for the first time the immunolocalization of chromogranin, ghrelin and gastrin immunoreactive cells in the stomach of sunda porcupine. However, in contrast with previous finding of crested porcupine and other rodents, the present result found serotonin immunoreactive cells in the fundic and pyloric gland regions and somatostatin immunoreactive cells in the gastric and fundic gland regions of sunda porcupine stomach. The differences may was caused by feed types, feeding habits and geographical locations of the species.

Key words: Sunda porcupine, gut endocrine cells, chromogranin, ghrelin, gastrin

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Corresponding Author: Teguh Budipitojo, Department of Anatomy, Faculty of Veterinary Medicine, Gadjah Mada University, 55281 Yogyakarta, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gastrointestinal endocrine cells were dispersed throughout the epithelia and gastric glands of the alimentary tract. These cells produce many biologically active polypeptides and amines, some of them hormones[1]. These hormones have important functions in the overall regulation of the digestive process, such as secretion of intestinal and associated glands, gut motility, nutrient absorption and intestinal blood flow[2]. The role of endocrine cells and their respective regulator peptides has attracted the interest of many investigators, who have described them in the gastrintestinal tract (GIT) of other vertebrates, such as amphibia[3], fish[4], birds[5] and mammals[6], seeking to establish the morphological role of these cells and respective peptides responsible for regulating numerous body functions.

The regional distribution and relative frequency of gastrointestinal endocrine cells has been found to vary with the animal species and feeding habits[2]. Fourteen types of endocrine cells have been demonstrated in the gastrointestinal tract of Tree Shrew[7] and Babirusa[8] by immunohistochemistry. Six types of endocrine cells have been shown in the malayan pangolin, manis javanica[9] and five in the barking deer[10]. These studies reveal interspecific differences and suggest correlation between endocrine cell distribution and feeding habits. This study aimed to analyze the existence and distribution of immunoreactive endocrine cells to chromogranin, ghrelin, somatostatin, serotonin and gastrin in the gastric mucosa of sunda porcupine (*Hystrix javanica*) using immunohistochemistry.

MATERIALS AND METHODS

Five stomachs of adult sunda porcupines, *Hystrix javanica*, about 67 cm in length, were purchased from a merchant in Tawangmangu, Central java, Indonesia and were used as samples. Stomach tissues of *Hystrix javanica* were fixed for 24 h in Bouin's solution, dehydrate in ethanol, cleared in xylene and embbeded in parrafin.

Stomach sections were cut serially in 4-5 µm thickenesses and stained by hematoxylin and eosin (HE) for conventional histological evaluation. For immunohistochemical staining of avidin-biotin peroxidase complex (ABC) methods[11] tissue sections were deparaffinized in xylene and rehydrated in decreasing series of ethanol concentrations. After washing in Phosphate Buffered Saline (PBS), endogenous peroxidase activity was blocked by incubating the section in H_2O_2 in methanol for 15 min. Primary antibodies of porcine chromogranin (Lot 8808014; Incstar, Stilwater, OK, USA) at 1:5,000 dilutions, ghrelin (bs-0467R; Bioss Inc. Massachusetts, USA) at 1:1000 dilution, serotonin (Sero-2-3; J Nishitsutsuji, Japan) at 1:5,000 dilution, gastrin (GP-1304; Yanaihara Institute, Japan) at 1:10,000 dilution and somatostatin (20H2T; Incstar, Stilwater, OK, USA) at 1:5,000 dilution were applied overnight at 4°C. To prevent non spesific staining, sections were incubate with nomal goat serum before incubation with the primary antibodies. The secondary antibody was applied for 45 min at room temperature. The immunoreactive site were visualized by tris-HCL buffer containing 3,3 diaminobenzidine tetrahydrocloride. Section were counterstained with mayer hematoxylin and examined with a conventional light microscope and photomicrographs were taken with digital camera.

The specificity of the immunohistochemical staining was confirmed by the replacement of primary antibody with normal rabbit serum. The semiquantitatif number of positif endocrine cell were graded subjectively into 4 classes as rare, few, moderate and numerous.

RESULTS

The present study revealed five kinds of endocrine cells immunoreactive for chromogranin, ghrelin, somatostatin, serotonin and gastrin in the gastric mucosa of sunda porcupine (*Hystrix javanica*). The chromogranin-positive cells were the most numerous, being more prevalent in the stomach glands. The other endocrine cells were identified in smaller numbers, some of them located in all regions or in specific regions.

The chromogranin immunoreactive cells were found rare, few and numerous in the gastric, fundic and pyloric gland regions, respectively (Fig. 1a-c). In all regions of the stomach, endocrine cells secreting ghrelin were detected rare, a weaker frequency compare with chromogranin (Fig. 1d-f).

The somatostatin immunoreactive cells were detected rare in the gastric and fundic regions (Fig. 2a, b), serotonin immunoreactive cells were found rare in fundic but few in pyloric regions (Fig. 2c, d), while gastrin immunoreactive cells were detected numerous in pyloric region of stomach of the sunda porcupines (Fig. 2e). Gastrin positive cell was also detected in the duodenal part of sunda porcupine, individually (Fig. 1f).

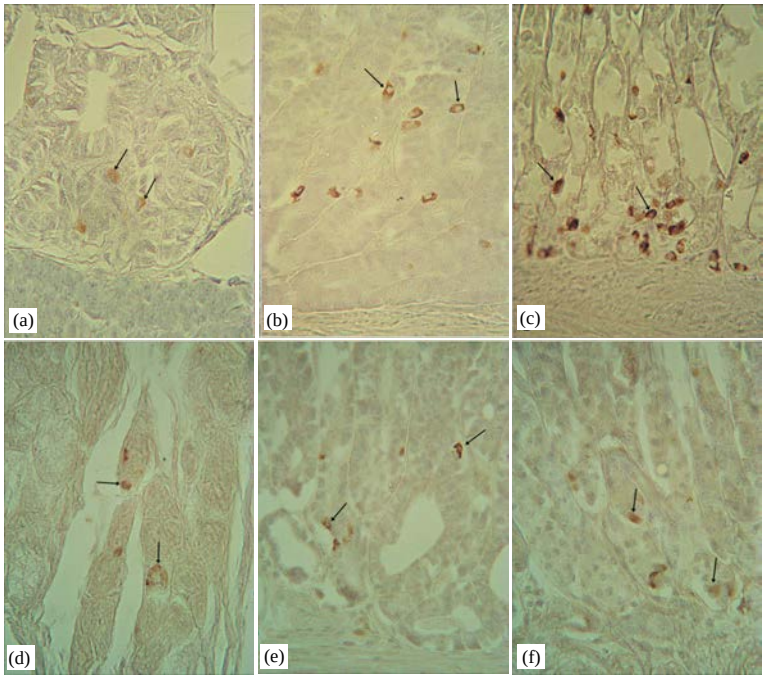

Fig. 1(a-f): Immonolocalization of chromogranin and ghrelin positive cells in the gastric, fundic and pyloric regions of the stomach of sunda porcupine (*Hystrix javanica*). As indicated by arrows, chromogranin cells were detected rare, few and numerous in the (a) Gastric, (b) Fundic, (c) Pyloric gland regions, respectively, while ghrelin cells were detected rare in (d) Gastric, (e) Fundic and (f) Pyloric gland regions of stomach of the sunda porcupines

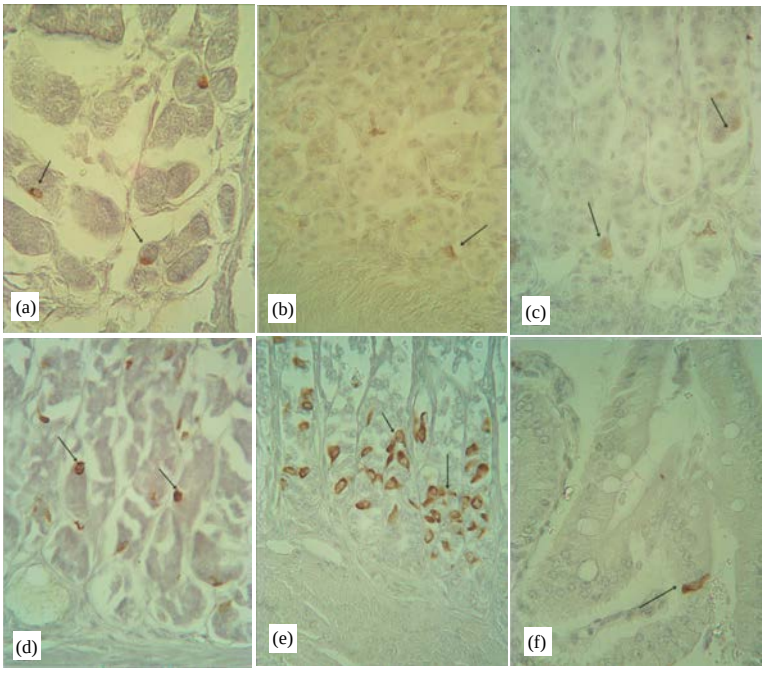

Fig. 2(a-f): Immonolocalization of somatostatin, serotonin and gastrin positive cells in the stomach of sunda porcupine (*Hystrix javanica*). As indicated by arrows, somatostatin cells were detected rare in the (a) Gastric, (b) Fundic gland regions, serotonin cells were found rare in (c) Fundic but few in (d) Pyloric gland regions, while gastrin cells were detected numerous in (e) Pyloric gland region of stomach of the sunda porcupines and (f) Gastrin positive cell was also detected in the duodenal part of sunda porcupine

DISCUSSION

Chromogranin has been used as a marker of endocrine cells as it is found in a wide spectrum of gut endocrine cells in mammals[12] and was detected in all of stomach gland regions of babirusa[8] and malayan pangolin[9]. In agreement with these previous findings, the present study found the presences of chromogranin immunoreactive cells in all gastric gland regions of sunda porcupine.

Ghrelin is a peptide hormone produced in the gastrointestinal tract which functions as a neuropeptide in the central nervous system[13]. Besides regulating appetite, ghrelin also plays a significant role in regulating the distribution and rate of use of energy[14]. Ghrelin immunoreactive cells are found mainly in the stomach[15]. In rodents, ghrelin immunoreactive cells were observed in all regions of the gastrointestinal tract and were most abundance in the mucosal layer of fundic gland region[16]. In agreement with these previous findings, the present study found ghrelin immunoreactive cells in all gastric gland regions of sunda porcupine without any differences on the density between gland regions.

Serotonin is secreted by the enterocromafin cells, widely distributed in the nervous system and in the gastro-entero-pancreatic endocrine cells[17], where it is used to regulate intestinal movements[18]. Serotonin immunoreactive cells were detected in the whole gastrointestinal tract of rat[19], mouse[20] and porcupine[21]. In the crested porcupine, serotonin immunoreactive cells were detected throughout the whole gastrointestinal tract and showed the highest frequencies in the cardiac region of stomach and duodenum[21,22]. In contrast with previous finding in crested porcupine and other rodents, the present result found serotonin immunoreactive cells in the fundic and pyloric gland regions of sunda porcupine stomach but not in the gastric gland region.

The somatostatin is a neuroendocrine hormone which was first isolated from the hypothalamus of the sheep[23] and plays important inhibition roles on the secretion of other neuroendocrine hormones[24]. Somatostatin immunoreactive cells were found predominantly in the pylorus gland region of manchurian chipmunk[25] and gerbil[26] stomach. In contrast with previous finding, somatostatin immunoreactive cells were not detected in the stomach of the crested porcupine[22]. In the present study, however, somatostatin immunoreactive cells were found in the gastric and fundic gland regions of sunda porcupine stomach but not in the pyloric gland region.

Gastrin is a peptide hormone produced by G cells of the pyloric antrum of the stomach and duodenum[27]. This hormone acts by increasing the force of antrum contractions and the constriction of the pyloric sphincter[28]. In agreement with ostrich[29] and malayan pangolin[9], the present study found gastrin immunoreactive cells in the gastric glands of the pyloric gland region and duodenum of sunda porcupine (*Hystrix javanica*).

CONCLUSION

The present study show for the 1st time the immunolocalization of chromogranin, ghrelin and gastrin immunoreactive cells in the stomach of sunda porcupine. However, in contrast with previous finding of crested porcupine and other rodents, the present result found serotonin immunoreactive cells in the fundic and pyloric gland regions and somatostatin immunoreactive cells in the gastric and fundic gland regions of sunda porcupine stomach. The differences may caused by feed types, feeding habits and geographical locations of the species.

SIGNIFICANT STATEMENT

The present study show for the first time the immunolocalization of chromogranin, ghrelin and gastrin immunoreactive cells in the stomach of porcupine, especially sunda porcupine (*Hystrix javanica*) which is endemic to Indonesia. In the previous report on crested procupine (*Hystrix cristata*) which is endemic to Italy, Sicily, North Africa and sub-Saharan Africa. Timurkaan *et al*.[21] and Yaman *et al*.[22] showed the presence of serotonin and somatostatin in the gastrointestinal tract. However, in contrast with previous finding of crested porcupine and other rodents, the present result found serotonin immunoreactive cells in the fundic and pyloric gland regions and somatostatin immunoreactive cells in the gastric and fundic gland regions of sunda porcupine stomach.

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