MORPHOLOGY OF THE GUT ENDOCRINE CELLS IN THE GASTROINTESTINAL TRACT OF THE LESSER MOUSE DEER *(Tragulus javanicus)*

MORFOLOGI SEL-SEL ENDOKRIN USUS DALAM TRAKTUS GASTROINTESTINAL KANCIL *(Tragulus javanicus)*

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ABSTRACT

The ultrastructure of endocrine cells in the gastrointestinal tract of the lesser mouse deer (*Tragulus javanicus*), the smallest ruminant, was studied using electron microscopy. The cells possess components such as rough endoplasmic reticulum, golgi complexes and mitochondria and characterized prominently by the presence of cytoplasmic secretory granules. The secretory granules were polymorphous, rounded, oval or spindle shaped and varied greatly in size and electron density from one cell type to another. The granules were generally concentrated in the intranuclear region of the cells. Two types of endocrine cells could be observed. Open type cells were oval, triangular or spindle in shape, showed apical luminal contact by means of microvilli. Closed type cells were generally round or triangular in shape. The endocrine cells were located in the basal portions, close to either capillaries or submucosal nerve fibers. The morphology of the endocrine cells was discussed in relation to their possible functions.

ABSTRAK

Penelitian ini menggambarkan ultrastruktur dari sel endokrin pada saluran pencernaan kancil, hewan ruminansia terkecil di dunia, dengan teknik elektron mikroskopis. Seperti sel-sel lain pada umumnya sel endokrin memiliki pula komponen-komponen sel seperti retikulum endoplasmik, badan golgi dan mitokondria. Yang paling khas pada sel endokrin adalah butir-butir sekretoris pada sitoplasma sel. Butir-butir ini sangat bervariasi dalam bentuk, ukuran serta densitas elektronnya antara sel endokrin satu dengan lainnya. Ada dua tipe sel endokrin yang dapat diamati. Sel endokrin tipe terbuka berbentuk oval, segitiga atau seperti gelendong dan bagian apikalnya dihubungkan dengan lumen oleh mikrovili. Sel endokrin tipe tertutup umumnya berbentuk bulat atau segitiga. Sel-sel endokrin tersebar diantara sel-sel eptelium atau kelenjar, cenderung berlokasi di daerah basal dan dekat dengan kapiler ataupun serabut syaraf. Morfologi sel endokrin yang ditemukan dibahas dalam kaitannya dengan kemungkinan fungsinya.

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INTRODUCTION

The gastrointestinal tract has been considered as the biggest endocrine organ in the body reference. It contains various endocrine cells that are dispersed in the epithelium throughout the length of the gastrointestinal tract. The gut endocrine cells has been known to play important roles in the regulation of overall digestive processes.

The gut endocrine cells are recognized ultrastructurally by the presence of cytoplasmic granules, which are the storage sites of the secretory products. The structure of these granules varies greatly from one endocrine cell to another and has therefore formed the basis for classification of these cells (Solcia *et al.*, 1987; Sundler and Håkanson, 1988; Polak, 1989). Furthermore, species differences in the morphology of the endocrine cells have also been noted (Sundler and Håkanson, 1988). The lesser mouse deer (*Tragulus javanicus*) is very interesting in the point of view of ruminant evolution. This animal is regarded as the smallest and the most primitive ruminant (Langer, 1988). An immunohistochemical study has been carried out in the lesser mouse deer and revealed the distribution and relative frequency of fifteen kinds of endocrine cells in the gastrointestinal tract (Agungpriyono *et al.*, 1994). However, the ultra structure of the endocrine cells in this animal has not been fully described in that study.

The present study was undertaken to describe the morphology of several endocrine cells in the gastrointestinal tract of the lesser mouse deer by a specific histochemical method and electron microscopy. The data obtained may support further immunocytochemical studies on the particular hormones produced by each gut endocrine cell based on their fine morphology and immunoreactivity.

MATERIAL AND METHODS

Three young adult *T. javanicus* animals weighing 1.5 - 2.1 kg were used in this study. Tissue samples were taken from certain portions of the abomasum, small and large intestines. For histological observations, tissues were fixed in Bouin's fluid, while for electron microscopy, small samples from certain portions as mentioned above were fixed in a mixture of 3% paraformaldehyde and 1% glutaraldehyde in 0.01M phosphate buffered solution (pH 7.3).

For light microscopy, tissue samples were dehydrated through an ethanol-xylene series and embedded in paraffin. Sections were cut serially at 5 μm thickness and mounted on glass slides. After deparaffinization, sections were stained using the Grimelius silver impregnation method (Grimelius, 1968) to demonstrate the endocrine cells. Slides were then dehydrated, cleared and mounted. Observations and photography were taken using a light microscope equipped with a camera unit.

For electron microscopy, after postfixation for 2 hours in 1% osmium tetroxide the samples were dehydrated in alcohol - propylene oxide series and embedded in spurr's resin. Ultrathin sectioning was performed using an ultramicrotome and sections were mounted on silver grids. Grids with sections were stained with uranyl acetate and lead citrate, observed and photographed using a transmission electron microscope (Hitachi, H-600) at an accelerating voltage of 100 kV.

RESULTS AND DISCUSSION

The present study using Grimelius staining method was able to demonstrate the gut endocrine cells in the gastrointestinal tract of the lesser mouse deer. The cells were distributed in all portions examined. The cells were intermingled with nonendocrine cells in the mucosa. In general, they were round or oval, triangular, long or slender in shape (Figs. 1A-C, 3C). Open type cells showing clear connection with the lumen could be observed in the pyloric gland region and the intestines, being more numerous in the latter. These cells were oval, triangular or spindle shaped. Closed type cells were generally round or triangular, some of them posessed basal cytoplasmic elongation. Closed type cells were numerous in the proper gastric gland region. In the small intestines, endocrine cells were distributed in the intestinal villi and intestinal glands (Fig. 1B). Those in the villi were mainly endocrine cells of the open type. In the large intestine, the endocrine cells were more numerous in the rectum (Fig. 1C).

Ultrastructural study using electron microscopy revealed fine morphology of some endocrine cells encountered in the sections of the samples (Figs. 2A-C, 3A-B). As a rule, the cells were equipped with cell components such as rough endoplasmic reticulum, golgi complexes and mitochondria. Each endocrine cell was characterized prominently by the presence of cytoplasmic secretory granules. The secretory granules varied greatly in size, shape and electron density from one cell type to another or even in one cell. The granules were round, polymorphous or oval and spindle shape and generally concentrated in the infranuclear region of the cells and normally absent from the apical portion. The electron density of the granules varied from weak to strong. In some endocrine cells, the core of the granules were surrounded by a clear halo. In these cells,

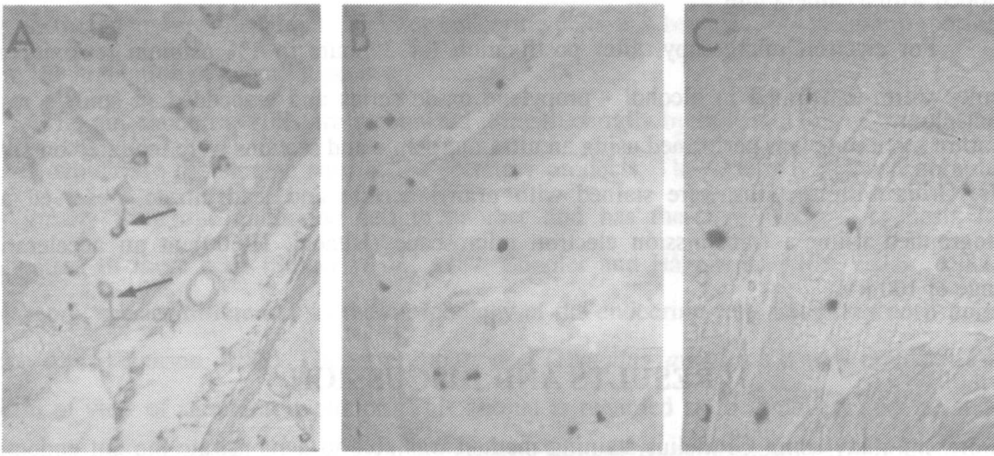

Fig. 1. Light micrographs of gut endocrine cells in the proper gastric gland (A), duodenum (B) and rectum (C). The endocrine cells are scattered among the epithelium cells of the mucosa. Some cells show a clear cytoplasmic elongation running along the basal membranes (*arrows*). *Grimelius stain*, A-C : x250.

electron cores were located either concentric or eccentric. Open type cells showed apical luminal contact by means of microvilli. Closed type cell were generally round or triangular in shape. The gut endocrine cells were mainly located in the basal portion. Closed type cells with basal cytoplasmic elongation, however, were not encountered in all sections examined.

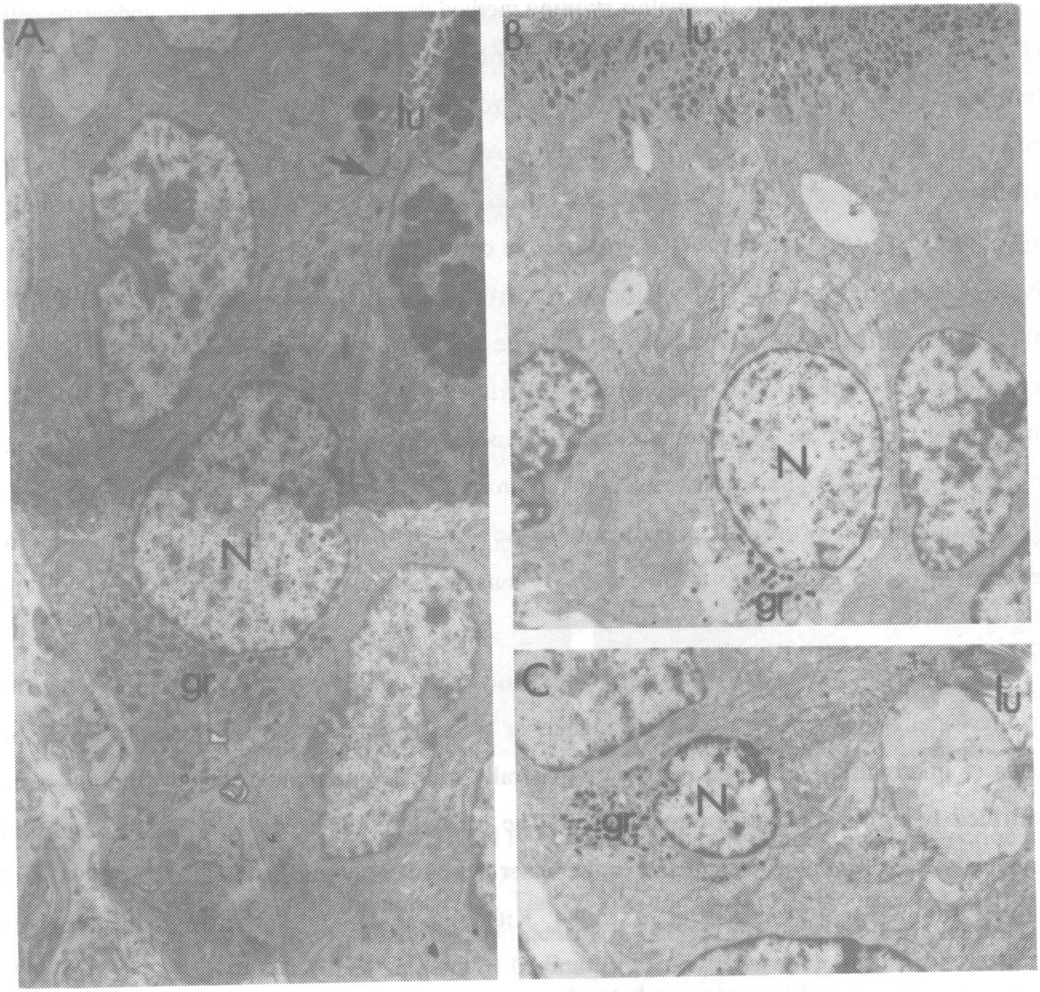

Fig. 2. Transmission electron micrographs of endocrine cells in the pyloric gland region (A) and jejunum (B, C). The endocrine cells are located in the basal portion. The cells show nuclei (N), golgi complexes, endoplasmic reticulums and specific secretory granules (gr) which are polarised towards the basal portions. The endocrine cell of A is an open type endocrine cell with round or slightly oval cytoplasmic secretory granules. The granules have core with weak to moderate electron density. The cell has luminal contact (*arrow*) with lumen (lu) by means of microvili. The cytoplasmic secretory granules of B are smaller, but show moderate to strong electron density and some are surrounded by clear halo. The endocrine cell in C has oval, round or elongated cytoplasmic granules with weak to strong electron density. A and C:x5,000; B : x4,000.

In the present study, Grimelius staining method demonstrated clearly the endocrine cells in the gastrointestinal tract of the lesser mouse deer by staining selectively the secretory granules. The silver impregnation could be divided into argyrophil and argentaffin reactions. The secretory granules of cells displaying argyrophil reactions retain silver ions from the impregnation, but visible metallic silver only appeared after slides being immersed in a reducing solution. Argentaffin cells, on the other hand, contain one or more chemical substances that can retain silver ions and reduce them into metallic silver (Grimelius, 1968; Grimelius and Wilander 1980; Polak, 1989). Most of the gut endocrine cells are argyrophil and show positive reaction with Grimelius silver impregnation staining method (Grimelius, 1968; Grimelius and Wilander 1980; Sundler and Håkanson, 1988), therefore positive endocrine cells observed in this study represent almost all population of endocrine cells in the gastrointestinal tract of the lesser mouse deer. As a rule, most of the endocrine cells, except those in the intestinal villi, were inclined to be located in the basal portions of the glands. This location is considered to give a maximum protection to the cells.

The endocrine cells were round, oval, spindle or triangular in shape. In this study, the open type cells were generally oval, triangular or spindle in shape having luminal contact by means of microvilli. The closed ones were generally round or triangular. These findings were confirmed ly light- and electron-microscopy. The present results coincide with the results of previous immunohistochemical studies in the lesser mouse deer (Agungpriyono *et al.*, 1994) and in other ruminants (Calingasan *et al.*, 1984; Kitamura *et al.*, 1985). These shapes may be characteristic for gut endocrine cells in all animal so far reported. The forms of endocrine cells are said to be well correlated with their characteristic functions. The open type cells are said to receive chemical stimuli from the gut lumen by the microvilli, while the closed ones receive stimuli by mechanical pressure from their surrounding area (Fujita *et al.*, 1988). The routes of secretion in the gut endocrine cells include endocrine, neurocrine and paracrine (Grube, 1986; Sundler and Håkanson, 1988; Fujita *et al.*, 1988).

In the present study, the endocrine cells were generally located in the basal portion, near capillaries and sometimes subepithelial nerve fibers. In the endocrine mode, the secretory hormones are released to the capillaries, while secretions to the subepithelial nerve plexuses is

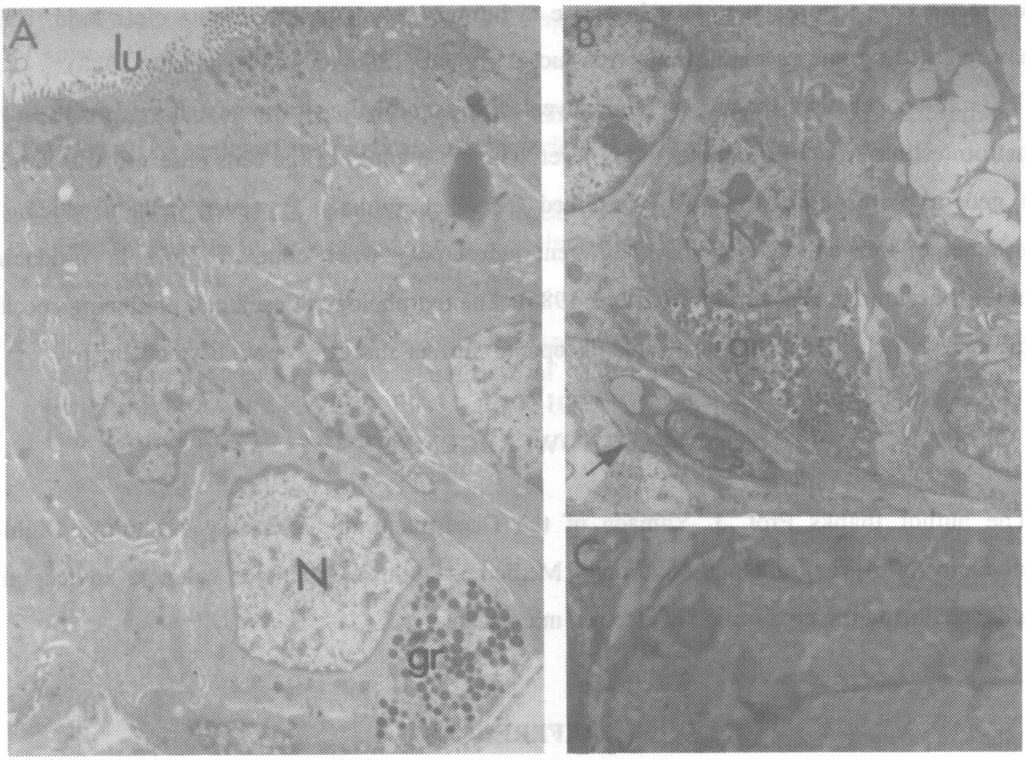

Fig. 3. Transmission electron (A and B) and light (C) micrographs of closed type endocrine cells in the rectum (A), colon (B) and proper gastric gland region (C). The cells has no luminal contact with the lumen (lu). The cytoplasmic secretory granules in A are round, with very weak to moderate electron density. Some granules are seen as clear vesicle. The cytoplasmic secretory granules of B are round, have strong electron density core surrounded by clear halo. The core are located eccentric within the granules. Nerve fibers *(arrow)* and fibroblast (s) are seen beneath the endocrine cell and the adjacent cells. A and B : x5,000; C : x500.

called neurocrine mode. Cells equipped with basal cytoplasmic processes are considered to have paracrine function as their secretions may influence the neighboring cells (Grube 1986; Sundler and Hakanson, 1988; Fujita *et al.*, 1988).

The presence of secretory granules was characteristic for gut endocrine cells. The electron density of the secretory granules in one cell varied from weak to strong. This is suggested to be correlated with the developmental stage of the granules within the cells. In gastrin producing cells, early granules are small electron-dense granules. Gastrin granules at progresive conversion stage of prohormone to hormone show a surrounding clear halo, while mature gastrin granules are large electron-lucent granules (Sundler and Håkanson, 1988).

In conclusion, the present study revealed fine morphology of some endocrine cells in the gastrointestinal tract of the lesser mouse deer. The morphology of the cells observed was similar in general with those previously described for other animals. However, cells producing a hormone in one animal may have different morphology when compared to cells producing similar hormone in other species (Grube, 1986). The morphology of each cell producing specific hormone in the lesser mouse deer remains topic to further study by immunocytochemistry.

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