Endocrine Cells of the Dog Gastrointestinal Mucosa

Ladislav Kubes, Karel Jirasek and Radovan Lomsky

Institute of Pathological Anatomy and First Medical Clinic,
Faculty of Medicine, Charles University,
Hradec Králové, Czechoslovakia

Received February 8, 1972

Introduction

The chromaffin cells of the gastrointestinal mucosa were described for the first time by Heidenhain (1870) in the gastric mucosa of the rabbit and dog. Subsequently, the chromaffin cells were found in the gastrointestinal tract of various animal species and they were designated by the names of their discoverers (Heidenhain cells, Kultschitski cells, Schmidt cells, Feyrter cells). In addition, these cells were also designated as the "enterochromaffin cells" (EC). It was not until 1952 that Erspamer and Asero identified the chromaffin substance of the secretory granules of the EC cells as 5-hydroxytryptamine (5-HT), which is a specific product of these cells.

The existence of other types of endocrine cells of the gastrointestinal tract was first mentioned by Kull (1913), who found the intestinal mucosa to contain non-chromaffin cells with small acidophilic granules, and by Erspamer (1939), who discovered non-argentaffin argyrophil cells in the gastrointestinal tract.


In addition to serotonin, which is a well-known product of the EC cells, gastrin, secretin, cholecystokinin-pancreozymin, glucagon-like substance and enterogastrone were established as the hormonal products of different parts of the gastrointestinal mucosa (Gregory and Tracy 1964, Jorpes and Mutt 1961, Sutherland et al. 1949, Unger et al. 1966, Brown et al. 1969).

The purpose of the present communication is to describe ultrastructural features of endocrine cells found in the gastric and intestinal mucosa of the dog.

Material and methods

Samples of the gastrointestinal mucosa were obtained from 4 dogs. The animals were anesthetized by an intraperitoneal injection of Thiopental and small pieces of tissue were taken from the cardia and fundus ventriculi, antral and pyloric mucosa, duodenal mucosa from the first, second and third portion of the duodenum,
from the first and middle portion of the jejunum and from the middle portion of the ileum.

Perfusion fixation with 2% glutaraldehyde in phosphate buffer, pH 7.4, was employed in two dogs. In the remaining two dogs, the tissue samples were fixed by immersion fixation using 5% glutaraldehyde in the same buffer. After post-fixation in 1% osmium tetroxide the tissue pieces were dehydrated and embedded in Vestopal W. The sections were double stained with uranyl acetate and lead citrate and examined with a Tesla 242E electron microscope.

Results

Five types of endocrine cells were found in the antro-pyloric mucosa: G cells,

![Image](image-url)

Figs. 1–4. 1, an antro-pyloric G cell reaching the gland lumen. Note the accumulation of the secretory granules at the base of the cell. ×7000. 2, a G cell in the uncinate process of the canine pancreas. Detail of the secretory granules with microfilamentous content. Note fusion of adjacent granules. ×26000. 3, detail of the secretory granules of a pyloric G cell. ×33000. 4, detail of the Golgi apparatus of a pyloric G cell. Osmiophilic prosecretory granules are seen in the neighbourhood. ×14000.
enterochromaffin-like cells, D cells, D₁ cells, and EC cells.

The G cells are the most numerous type of the endocrine cells in the antro-pyloric mucosa. They are situated predominantly in the middle layer of the mucosa among mucoid and mucous-neck cells. The G cell shows a pyramidal form (Fig. 1), the basis of the cell is in contact with the basement membrane of the pyloric gland, the apical portion reaches the lumen of the gland and is covered by microvilli. The
apical portion of the cell contains a well-developed Golgi complex with dark secretary progranules (Fig. 4), further lamellae of the endoplasmic reticulum, lysosomes and elongated mitochondria. The nucleus of the G cell shows a typical multilobate form. The basal portion of the cell is packed with mature secretary granules. The secretary granules show a continuous limiting membrane and fibrillar content of low electron density (Fig. 3). The neighbouring sacs may at times have a tendency to fusion. The diameter of the secretary granules ranges from 200 to 530 nm.

The enterochromaffin-like cells (ECl cells) are scattered along the entire length of the canine gastric mucosa. In the antro-pyloric mucosa they are situated mainly in the middle third of the glands similarly as the G cells, whereas they display no distinctive localization in the other parts of the gastric mucosa.

The ECL cell exhibits a pyramidal form. The basis of the cell reaches the basement membrane, the apical portion extends up to the gland lumen and is covered by microvilli (Fig. 6). The cell exhibits a light cytoplasm with a small
number of free ribosomes and scarce endoplasmic reticulum. The nucleus of the cells is round. The Golgi complex and the mitochondria are located in the apical portion of the cell. The basal portion of the cytoplasm contains secretory granules of two types. The first type is represented by small round granules with an osmiophilic core which is separated from the limiting membrane by a light narrow halo, whereas the second type showed granules with a dark homogeneous core (diameter 140–270 nm) located eccentrically in larger light sacs (diameter 230–470 nm) (Fig. 5).

Figs. 10–11. 10, a D1 cell at the base of a pyloric gland. The cytoplasm in the basal portion of the cell is filled with secretory granules containing light vacuoles. ×16,000. 11, secretory granules of a D1 cell with an osmiophilic granular core, which is detached from the limiting membrane by a light halo. ×34,000.

The D cells (Fig. 7) were found in the antro-pyloric mucosa, duodenal mucosa, and occasionally also in the jejunum. The cytoplasm of these cells contains numerous microfilaments and elongated mitochondria. The secretory granules of the D cells are of intermediate electron density and markedly granular, and the limiting membrane is closely adjacent to the granule core (Fig. 8). The diameter of the secretory granules amounts to 160–360 nm.

The D1 cells are situated in the glands of the cardia, fundus and pylorus with
a maximal prevalence in the pylorus. These small cells are lying on the basement membrane of the glands. The cytoplasm contains very numerous microfilaments and clear vacuoles in addition to secretory granules (Fig. 10). The secretory

Figs. 12–13. 12, an enterochromaffin cell with distinctly elongated osmiophilic secretory granules. Note the homogeneous structure of the granules. The limiting membrane is separated from the dark core by a light space. ×23,000. 13, an EC cell with irregular less elongated secretory granules. The granules show a finely granular structure and a closely-applied limiting membrane. ×24,000.
granules (diameter 130–250 nm) display finely granular osmiophilic core, which is detached from the limiting membrane by a narrow clear space (Fig. 11).

The enterochromaffin cells (EC) are the most numerous endocrine cells in the gastrointestinal mucosa of the dog. They are situated in the stomach, duodenum, jejunum and ileum. These cells are most numerous in the first portion of the duodenum at the base of the glands. The apical portion of the light cytoplasm contains the Golgi complex and occasional mitochondria. The characteristic feature of the EC cells are irregular, highly osmiophilic secretory granules in the cytoplasm.

The secretory granules of the gastric EC cells are smaller (diameter 120–360 nm) than those of the intestinal EC cells (diameter 120–480 nm).

The limiting membrane of EC cells with less elongated secretory granules is in close contact with the adjacent finely granular osmiophilic core (Fig. 13) whereas the limiting membrane of distinctly elongated secretory granules leaves a light space around the homogeneous dark core (Fig. 12). Since these findings were made on material obtained by perfusion and both these cell forms were found in identical

Fig. 14. An X cell in the cardiac mucosa. The light cytoplasm contains numerous homogeneous osmiophilic secretory granules with a closely-applied limiting membrane. ×24,000.
Figs. 15-17. 15, an A-like cell in the cardiac mucosa. The secretory granules show a dark core and a distinct light halo. ×23,000. 16, secretory granules of an A cell of the canine islet of Langerhans. ×23,000. 17, an S cell in the first portion of the duodenum. The secretory granules show an irregular osmiophilic core and light halo. ×36,000.
material, artificial changes seem to be ruled out.

The mucosa of the fundus and cardia was found to contain, in addition to the ECL cells, D₁ cells and EC cells, two types of endocrine cells, i.e., the X cells and A-like cells.

The X cells are located in the mucosa of the cardia and fundus without any marked maximum. The light cytoplasm contains highly osmiophilic homogeneous secretory granules with a closely adjacent limiting membrane (Fig. 14). The diameter of the granules ranges from 250 to 380 nm. Occasional ribosomes are scattered throughout the cytoplasm and the apical portion of the cells contains a well-developed Golgi apparatus.

Figs. 18-19. 18, an I cell in the first portion of the duodenum. Note the accumulation of the secretory granules at base of the cell. ×17,000. 19, secretory granules of an I cell. Note the osmiophilic core with a closely-applied limiting membrane. ×35,000.

The A-like cells are small cells lying on the basement membrane. The characteristic feature of these cells are their secretory granules with a highly osmiophilic round core, which is separated from the limiting membrane by a marked band of clear space (Fig. 15). The diameter of the granules ranges from 270 to 340 nm.

The mucosa of the duodenum, jejunum and ileum was found to contain S cells, I cells, D cells, L cells, EC cells and cells with secretory granules possessing a dark core and a distinctly granular structure.

The S cells are situated predominantly in the first portion of the duodenum and their number declines markedly in the further course of the intestinal tract.
Figs. 20-21. 20, an L cell in the ileum. Large, osmiophilic, homogeneous secretory granules. ×35,000. 21, a new cell type in the jejunal mucosa. The cell shows granular secretory granules, the centre of which is more osmiophilic. ×35,000.
Contrary to the EC cells, the S cells are located more superficially. The secretory granules of the S cells show a highly osmiophilic irregular core, which is separated from the limiting membrane by a light halo (Fig. 17). The diameter of the secretory granules varies from 120 to 210 nm.

The I cells are lying in the glands of the duodenum, jejunum and occasionally also of the ileum. The secretory granules of these cells are concentrated in the basal portion of the cell (Fig. 18). The secretory granules of the I cells have an osmiophilic homogeneous core, which is in close contact with the limiting membrane (Fig. 19). The diameter of the secretory granules is 170 to 310 nm. The apical portion of the cell harbors the Golgi apparatus and occasional lamellae of the endoplasmic reticulum.

The L cells are located in the mucosa of the ileum both in the glands and in the villi without any distinct maximum. Their number decreases progressively in the upper part of the intestinal tract. These cells are typical by large round secretory granules (diameter 180 to 520 nm). The granules show homogeneous highly osmiophilic core and a closely attached limiting membrane (Fig. 20).

In the duodenum and jejunum we found occasional previously undescribed cells, the secretory granules of which show a distinctly granular structure and a more osmiophilic center. The limiting membrane is in close contact with the core (Fig. 21). The diameter of the granules is 150 to 300 nm.

Discussion

Cells assumed to be producing gastrin were designated as the G cells by Solcia et al. (1967). In the original concept of these authors the population of the G cells included all non-EC endocrine-like cells of the antro-pyloric mucosa. However, from the present point of view, the G cells of Solcia et al. (1967) comprise the G cells proper as well as other cells with ultrastructural characteristics different from those of the gastrin-containing G cells. The ultrastructure of the G cells proper of the rat was described by Forssmann et al. (1969) who designated this cell as their cell type V. Similar findings were reported by Vassallo et al. (1969) for the cat and by Pearse et al. (1970) for the man. All these authors stress the ultrastructural differences between the G cells of the antro-pyloric mucosa and the D cells of the islets of Langerhans.

On the contrary Solcia et al. (1969) describe the G cells of the canine antro-pyloric mucosa as a cell type similar to the D cells of the islets. In the present study, the G cells of the antro-pyloric mucosa of the dog showed marked differences from the D cells of the canine pancreatic islets and their ultrastructure coresponds to the G cells of the dog uncinate process (Forssmann 1970, Jirásek et al. 1973), which are probably identical with the X cells (Bencosme and Liepa 1955, Lazarus and Shapiro 1971) and F cells (Solcia et al. 1969, Forssmann and Orci 1969).

The localization of the G cells in the middle third of the antro-pyloric mucosa corresponds to the maximal concentration of gastrin found in this zone by Bromé et al. (1968), McGuigan (1968) and McGuigan and Greider (1971) first employed the immunofluorescent and immunoenzyme technique to establish the cellular
origin of gastrin in the human and porcine antro-pyloric mucosa. These investigators found the gastrin containing cells irregularly interspersed along the antral glands with the maximum in the middle third of the glands.

A direct proof of the presence of gastrin in the antro-pyloric G cells was obtained by the aid of the immunoelectron microscopic technique using peroxidase-labeled antibodies (Lomský, Jirásek and Kubes 1972). The presence of immunoreactive gastrin was found in the cytoplasm of these cells, whereas the secretory granules were completely negative. This finding is in agreement with the hypothesis of Forssmann and Orci (1969) regarding the unusual mode of secretion of the G cells. However, the identification of the gastro-duodenal G cells with Davenport positive cells (Lomský et al. 1971) is incorrect and will be revised.

The ECL cells were identified as an independent cell type only by electron microscopic studies. Some investigators assumed these cells to produce catecholamines because of the similarity of these cells with those of the adrenal medulla (Forssmann et al. 1969), but catecholamines have not been found in the ECL cells (Håkanson et al. 1970, Capella et al. 1971). These results are supported by the fact that the ECL cells are resistant to the action of reserpine (Capella et al. 1971, our observations). The presence of these cells in the fundic and antro-pyloric mucosa corresponds to the distribution of non-argentaffin argyrophil cells described by Håkanson and Owman (1966). The cells show a high content of DOPA-decarboxylase (Håkanson, Lilja and Owman 1967) and in mice and rats these cells contain histamine (Håkanson and Owman 1967). In other animals including the dog histamine is present only in the mast cells (Aures et al. 1968). Håkanson et al. (1970) described a striking correlation between the localization of the ECL cells and the concentration of the vitamin B₁₂ binding proteins. Håkanson's hypothesis was questioned by most authors studying the ultrastructure of this cell type (Vassallo et al. 1969, Forssmann et al. 1969), who are of the opinion that the ECL cells do not reach the lumen of the glands. However, findings presented in this study support the hypothesis of Håkanson, because the ECL cells were found to reach the gland lumen and to be covered by microvilli. The secretory granules of the ECL cells are well contrasted with phosphotungstic acid (Capella et al. 1971). According to Silverman and Glick (1969) this fact is due to the presence of cationic groups of polypeptides or proteins. Thus, the secretory product of this cell type remains to be established. Solcia et al. (1970) found proliferation of the ECL cells in patients with gastritis and suggest that this cell type may give rise to some gastric carcinoid tumours.

In the dog, the ultrastructure of the antro-pyloric D cells is closely similar to that of the D cells of the islets of Langerhans. The diameter of the secretory granules of the antro-pyloric D cells ranges from 160 to 360 nm and that of the islet D cell, from 220 to 370 nm. The structure of the secretory granules is identical in both cell types. After administration of 0.1 N HCl into the canine stomach, Fujita and Kobayashi (1971) observed an emiocytic release of the secretory granules in the D cells and suggested that this cell type produces a hormone inhibiting the gastric acid secretion, such as secretin.

According to some authors (Solcia et al. 1970, Pearse et al. 1970, Vassallo
et al. 1971), the gastric D cells and X cells (Vassallo et al. 1969) of the cat, dog, pig and man represent one cell type. However, the present findings show distinct differences between the canine D cells and X cells and the two cell types appear to represent two entirely different populations of endocrine cells. The secretory granules of the D cells show a low electron density, they are markedly granular and possess a distinct limiting membrane. On the other hand the X cells show larger highly osmiophilic and homogeneous secretory granules with an indistinct limiting membrane. The D cells are situated predominantly in the pyloric antrum, the pylorus and the duodenum, whereas the X cells show a maximum in the fundus and cardia. The hormonal product of the X cells is unknown.

The term the D1 cell was first employed by Vassallo et al. (1971) and Capella et al. (1971). According to these authors these cells occur in the pyloric and fundic mucosa of man, cat, dog and pig. They are distinctly different from the D cells proper. Our findings in the gastric mucosa of the dog are the same as those of the Italian authors, but the D1 cells were found also in the cardiac mucosa.

A specific product of the EC cells is serotonin (Erspamer and Asero 1952, Feldberg and Toh 1953). Vassallo et al. (1969) distinguished two different structures in the secretory granules of the EC cells, i.e., highly osmiophilic argentaffin internal bodies, which contain probably serotonin, and a less osmiophilic substance of presumably protein nature, which forms the remaining part of the granule. These results might correspond to particular forms of carcinoid tumours that produce serotonin as well as other substances such as the callicrein-like and prostaglandin-like substance (Oates et al. 1964, Sandler et al. 1968).

Our material showed differences in the ultrastructural characteristics of the EC cells. On the basis of these differences it is possible to divide the EC cells into two groups. EC cells of the first group show elongated homogeneous secretory granules and the limiting membrane of these granules is separated from the dark core by a light space. EC cells of the second group show less elongated secretory granules with a finely granular core and a closely-applied limiting membrane. It remains to be established, whether the differences between the two groups of EC cells represent different developmental stages or secretory cycles of the EC cells, or whether the EC cells constitute a heterogeneous population of cells with different functions. The latter possibility seems to be supported by the observations of Håkanson et al. (1969), who divided the EC cells into two groups (i.e., reserpine-resistant and reserpine-sensitive) according to their sensitivity to reserpine.

The ultrastructure of the A-like cells is closely similar to the ultrastructure of the A cells of the canine islets of Langerhans. Sutherland et al. (1949) found a glucagon-like substance in the canine fundic mucosa and their findings were further extended by Unger et al. (1966). On the basis of these results Orci et al. (1968) and Forssmann et al. (1969) suggested that the A-like cells produce "enteroglucagon". This substance may show an immunologic cross-reaction with antisera to pancreatic glucagon, but the molecular weight and the biological effect of these two substances differ. In the dog, the glucagon-like immunoreactivity (GLI) was found in the mucosa of the fundus, jejunum and ileum (Unger et al. 1968). Polak et al. (1971) found the GLI in some cells of the fundic and small intestinal glands
with the use of the immunofluorescent technique. According to these authors, the immunoreactive glucagon-like substance is present in the fundic A-like cells and in the small intestinal L cells.

Polak et al. (1971) and Bussolati et al. (1971) suggested the canine S cells to be the site of origin of secretin. The conclusions of these authors are based on a close correlation between the localization and frequency of the S cells and that of the immunoreactive cells found with the use of the immunofluorescent technique.

The I cells were first described by Bussolati et al. (1971), who separated this cell type from the population of the L cells. The product of this cell is unknown, but the localization of the I cells seems to correspond to the distribution of cholecystokinin-pancreozymin in the intestinal mucosa (Harper and Raper 1943). Our finding of the maximal incidence of the L cells in the ileum corresponds to the results of Bussolati et al. (1971). The L cells show argyrophilia with the Davenport and Grimelius techniques (Pearse et al. 1970, Bussolati et al. 1971) and differ in this way from the gastric X cells and intestinal I cells, which show similar ultrastructural characteristics. In addition, the L cells stain well with lead haematoxylin (Bussolati et al. 1971) whereas the X cells do not stain with this technique (Vassallo et al. 1969). Polak et al. (1971) regard the L cells as the site of origin of the glucagon-like immunoreactive substance.

It remains to be established whether the cell type with granular secretory granules and an osmiophilic core represents an independent cell type or a developmental stage of the D or I cell, respectively.

Summary

Endocrine cells in the gastrointestinal tract of the dog have been studied by electron microscopy.

Seven types of endocrine cells were found in the gastric mucosa: the gastrin-producing G cells, serotonin-producing enterochromaffin cells, enterochromaffin-like cells, A-like cells, D cells, D̆ cells, and X cells.

The enterochromaffin cells may be subdivided into two cell types according to the shape and structure of the secretory granules. Contrary to previous reports, the enterochromaffin-like cells were found to reach the gland lumen. Implications resulting from this observation are discussed. The X cells and D cells have so far been described as one single cell type. According to the present findings, the two cells differ distinctly in several respects and should be regarded as two separate cell types.

The intestinal mucosa was found to contain the enterochromaffin cells, S cells, I cells and L cells. In addition, we observed an heretofore undescribed cell type, the secretory granules of which show a granular structure and a highly osmiophilic core. Since we were unable to find any transitory forms between these cells and established cell types, this cell type may represent a new element in the population of endocrine cells of the gastrointestinal mucosa.
References

Aures, D., Håkanson, R., Ownman, Ch. and Sporrong, B. 1968. Cellular stores of histamine and monoamines in the dog stomach. Life Sci. 7: 1147-1153.


— and Asero, B. 1952. Identification of enteramine, the specific hormone of the enterochromaffine cell system, as 5-hydroxytryptamine. Nature (Lond.) 169: 800-801.


Jorpes, J. E. and Mutt, V. 1961. The gastrointestinal hormones, secretin and cholecystokinin-


— and Greider, M. H. 1971. Correlative immunochemical and light microscopic studies of the gastrin cell of the antral mucosa. Gastroenterology 60: (2); 223-236.


