An Electron Microscope Study on a Neuro-Endocrine Complex in the Proventricular Mucosa of the Finch, *Uroloncha striata var domestica*

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*Summary.* An endocrine complex consisting of endocrine cells, nerve endings, unmyelinated nerve fibers and Schwann cells is found near blood capillaries in the lamina propria of the proventricular mucosa of the finch.

At least two types of endocrine cells (type I and II) are present mainly in the lamina propria mucosae where they form a neuro-endocrine complex, but some cells of both types are also seen in the basal part of the epithelial layer like mammalian basal-granulated cells. The type I cell has large round secretory granules of uniform ultrastructure. The type II cell contains small granules whose content is fairly diverse in density and is surrounded by either closely fitted or a vacuolated limiting membrane.

The nervous elements and endocrine cells of the complex are enclosed by a common basal lamina. Unmyelinated nerve fibers are encircled by either Schwann cell or endocrine cell cytoplasm. Most nerve endings, which have small clear vesicles and large granular ones, are thought to be cholinergic. Some of these make synaptic contact with both types of endocrine cells. Probable adrenergic endings containing small cored vesicles as well as large granular ones are very few in number and come into contact with the type I endocrine cells. These findings indicate a nervous regulation of the endocrine function in the finch proventricular mucosa.

The peculiar, subepithelial position and the intimate relation to nervous elements of the finch proventricular endocrine cells are discussed in comparison with the pancreatic islet cells.

Electron microscopic studies during recent years reveal up to 9 different types of endocrine cells in the mammalian gastro-enteric mucosa (Forssmann et al., 1969; Capella et al., 1969; Vassallo et al., 1969, 1971; Kobayashi et al., 1970, 1971; Pearse et al., 1970; Sasagawa et al., 1970, 1973; Osaka et al., 1971). As Fujita (1973) has proposed, it is reasonable to consider the hormones and their source cells in the gut mucosa and pancreatic islets as a single family, GEP (Gastro-Entero-Pancreatic) endocrine system for the following reasons: both tissues share some types of endocrine cells, some of their hormones are closely related in structure and nature, some kinds of pancreatic hormones have a marked effect on the gut, some pancreatic tumors produce typical gut hormones, and the pancreas originates embryologically from the gut mucosa.

It has been known that the pancreatic islets are controlled by the autonomic nervous system as well as by the blood glucose level. Many electron microscopic studies reveal the nervous element in the islet tissue (Bencosme, 1959; Winborn, 1963; Stahl, 1963; Legg, 1967; Esterhuizen et al., 1968; Watari, 1968; Kobayashi...
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and Fujita, 1969; Shorr and Bloom, 1970; Watari et al., 1970; Kern et al., 1971; Klein, 1971). However in the gastro-enteric mucosa, the innervation of endocrine cells has never been reported.

The present author found a close relationship between nerve fibers and endocrine cells in the proventricular mucosa of the finch. This structure which deserves the designation of neuro-endocrine complex will be reported in the present paper.

**Materials and Methods**

Several finches, Urolochon striata var domestica, of both sexes fed ad libitum were used for this study. After anesthesia with ether, each bird was perfused from the left ventricle of the heart with 2.5% glutaraldehyde buffered with phosphate at pH 7.4. The same fixative was injected into the lumen of the proventriculus. Small tissue blocks were obtained from the proventriculus, fixed with 2.5% glutaraldehyde and postfixed with 1% osmium tetroxide. After dehydration through graded concentrations of ethanol, they were embedded in Epon epoxy resin. Thin sections were doubly stained with uranyl acetate and lead acetate and examined in a Hitachi HU-11D electron microscope.

**Results**

The proventriculus, or the glandular stomach of the bird has characteristic compound glands consisting of highly developed secretory tubules which open into secretory ducts and finally into the lumen of the proventriculus. Two types of exocrine cells are found in the finch proventricular mucosa: surface mucous cells lining the mucosal surface and secretory ducts, and oxyntic cells forming secretory tubules.

The endocrine cells are chiefly located near the orifice of the secretory tubule. At least two types of endocrine cells, types I and II, are identified in the finch proventriculus by the ultrastructural characteristics of the secretory granules (Fig. 1).

**Type I cells**

Most type I cells are ovoid. The nucleus is also oval and has a prominent nucleolus (Fig. 3). A bundle of filaments is frequently seen in the nucleus (Fig. 2b).

The rough endoplasmic reticulum is moderately developed. Some cisternae make parallel arrays and others are scattered among secretory granules. The Golgi complex is well developed and formation of secretory granules is seen in the complex (Fig. 3).

Secretory granules are round, homogeneously electron dense, and 350-550 μ in diameter (Fig. 1a). The clear space between their content and the surrounding limiting membrane is very narrow or hardly recognizable. A type I cell may sometimes have larger and slightly less dense granules than an adjacent cell (Fig. 1a).

In general, the former cell contains more numerous secretory granules than the latter. The localization and the relation to nervous elements are the same. This study could not clarify whether these morphologically slightly different cells represent two different cell types or different aspects of the same cell type plus possible artifacts.

A cilium is sometimes found near the Golgi area (Fig. 2b).
Type II cells

Type II cells are elongated or irregularly shaped. The nucleus is ellipsoid. In some sections the cytoplasm seems scanty with poor membranous structures. In the other sections the rough endoplasmic reticulum and the Golgi apparatus are moderately developed and mitochondria are fairly numerous. Figures suggesting formation of secretory granules are frequently seen in the Golgi complex. Secretory granules are numerous in the peripheral cytoplasm, especially in the cytoplasmic process. They show considerable variation in electron density and in

Fig. 1. a. Three varieties of type I cells (A, B, C). Note the different size and electron density of their secretory granules. Ca blood capillary. b. A part of a type II contact with an oxyntic cell (O). The secretory granules vary in electron density, some being vacuolated. a: $\times 12,000$, b: $\times 20,000$
Fig. 2.  

a. A type I cell in contact with an oxyntic cell (O). The endocrine cell membrane does not face the intercellular secretory canaliculus (S) between the oxyntic cells. 

b. A type I cell in lamina propria. Two nerve endings (E) are in direct contact with the cell and nerve fibers (arrows) are associated with Schwann cells. A bundle of filaments (F) is seen in the nucleus and a cilium (C) is found near the Golgi area. Ca blood capillary.  

a: $\times 11,000$,  
b: $\times 8,500$
shape (Fig. 1b). Most secretory granules have solid and dense, or finely granulated and moderately dense contents which are surrounded by limiting membranes leaving a narrow or hardly recognizable space. Light granules containing a flocculent material and vacuolated granules containing a small dense core are frequently intermingled.

A cilium is sometimes seen near the Golgi area as seen in type I cells.

**Localization of endocrine cells and the neuro-endocrine complex**

Both types of endocrine cells are found in the lamina propria as well as in the epithelium in the proventricular mucosa (Fig. 2).

In the epithelial layer of the gland and its secretory duct, isolated endocrine cells are scattered in contact with surface mucous and oxyntic cells (Fig. 2a). The basal cell membrane of the endocrine cell faces the basal lamina of the epithelium but the cell apex does not reach the lumen of the gland. Thus, this cell has a characteristic common with the mammalian endocrine cells of the closed type (FUJITA and...
Secretory granules are gathered in the basal cytoplasm. Desmosomes are sometimes found between an endocrine and an exocrine cell.

The endocrine cells are far more numerous in the lamina propria than in the epithelium (Fig. 2b, 3). Isolated or small groups of endocrine cells are present in contact with nerve fibers and Schwann cells near blood capillaries and they form a neuro-endocrine complex surrounded by a basal lamina. Unmyelinated nerve fibers, either naked or enclosed by the Schwann cytoplasm, are adjacent to the endocrine cell or encircled by it.

Nerve endings in the complex can be divided into two types depending on the structure of the synaptic vesicles (Fig. 4). Most nerve endings have small clear vesicles of about 500 Å in diameter and large granular vesicles of 1,200 Å in diameter. A few endings have small cored vesicles of 500 Å in diameter in addition to the large

![Image](image_url)

**Fig. 4.** a. and b. A nerve ending of cholinergic (a) and adrenergic (b) type in direct synaptic contact with type I cells. c. A type II cell is associated with many nerve endings of cholinergic type (E), nerve fibers (F) and a Schwann cell (S). Thickening of pre- and post-synaptic membranes is seen in a synapse (arrows). a: ×25,000, b: ×25,000, c: ×20,000
granular ones. Some of the former type endings make direct synaptic contacts with type I as well as type II endocrine cells (Fig. 4a, c) and a thickening of pre- and post-synaptic membranes is sometimes found. Others are separated from the endocrine cell by the thin cytoplasm of the Schwann cell and face the basal lamina (Fig. 4c). The latter type endings containing small cored vesicles are in contact with type I cells without any membrane thickening in some cases (Fig. 4b) or face the basal lamina in other cases.

Numerous nerve fibers might end on a single endocrine cell making synaptic contacts.

Discussion

By summarizing their previous studies and considering the agreement in the symposium on "The Origin, Chemistry, Physiology of the Gastrointestinal Hormones" held in 1969 in Wiesbaden, SASAGAWA et al. (1973) classified the GEP endocrine cells into the following 10 types; EC (enterochromaffin), EC-like, G (gastrin-producing), D (and D-like), L (large granules), S (small granules), M (medium-sized granules), T (tentacle-like microvilli), B and A (and A-like) cells. Electron microscopically, type II cells of the finch proventriculus resemble mammalian EC-like cells. Type I cells seem to correspond to one or two of the L, M, A-like and D-like cells. It would need extensive morphological, physiological, biochemical and immunological studies to decide which types of mammalian endocrine cells correspond to the bird ones.

It is well known that chemical information, such as luminal pH and arrival of specific substances, controls the secretion of gastrointestinal hormones. As to the control mechanism of the gastrin secretion, REDFORD and SCHOFIELD (1965) postulated excitatory and inhibitory nervous connections between the receptor in the antral mucosal surface and gastrin secreting cells via the Meissner's plexus. However, their pharmacological study using local anesthetics failed to find any support for the existence of an inhibitory nervous mechanism. Moreover morphological studies have revealed neither the receptor nor nervous connections. FUJITA and KOBAYASHI (1971) and KOBAYASHI and FUJITA (1973), classifying the GEP endocrine cells into open and closed types as to whether the cell reaches the gut lumen or not, consider the open type cell a recepto-secretory cell which receives chemical information from the lumen through the cell apex and controls the hormone secretion by itself. With this hypothesis, they demonstrated D-like cell degranulation by lowered luminal pH (FUJITA and KOBAYASHI, 1971), G cell degranulation by elevated pH and EC cell degranulation by hypertonic glucose administration into the lumen (KOBAYASHI and FUJITA, 1973). As for the control of the closed type cell, FUJITA and KOBAYASHI (1973) suggested the influence of humoral stimuli as in the pancreatic islet cells whose hormone secretion is regulated by the blood sugar level. Endocrine cells of the finch proventriculus are closed in type similar to those of the human gastric corpus.

The present study demonstrated two types of nerve terminals. The main type terminal containing small clear and large granular vesicles is regarded as cholinergic and the occasional type having small cored vesicles as well as large granular ones as adrenergic according to the criterion of RICHARDSON (1964). Thus both the cholinergic and adrenergic terminals form synaptic contacts with the finch proventricular endocrine cells. Among the GEP endocrine system, the autonomic innervation of
pancreatic islet cells has been studied as cited above and the A, B and D cell each may receive terminals of both adrenergic and cholinergic types (Kobayashi and Fujita, 1969).

The present study further indicates that the endocrine cells of the finch proventriculus are in contact with such rich axonal and Schwann elements that the designation of neuro-endocrine complex seems to be most suited. An apparently homologous structure is again found in the pancreatic islet whose innervation may be very rich and in some animals represents a conglomeration of nervous and islet cell elements called a neuro-insular complex (Fujita, 1959; Kobayashi and Fujita, 1969, Watari, 1973).

The present findings demonstrate a conspicuous instance of the possibility that closed type GEP endocrine cells, in contrast to the open type cells whose direct contact with nerve terminals has been revealed by none of the previous studies, may be directly supplied by the synapses of the autonomic nervous system. It seems worthwhile to search for a possible innervation of the closed type endocrine cells in the gastro-enteric wall in other animals including man.

A further analogical situation of the endocrine cells of the finch proventriculus and the pancreatic islet cells is found in their main position enclosed in the connective tissue apart from the gastro-enteric epithelium. This localization of the proventricular endocrine cells recalls the long disputed question concerning the origin and development of the enterochromaffin cell system, i.e., the mesenchymal, nervous or entodermal epithelial origin. Recently, Pearse (1969) proposed the concept of an “APUD series” for endocrine cells capable of Amine and Precursor-Uptake and Decarboxylation and of polypeptide production. The GEP endocrine cell is one of the most important members of the APUD series together with the thyroid C cells and adrenal medullary cells. According to the view of Pearse (1969) the cells of the APUD series originate from neural crest and migrate into different organs. The close relationship between endocrine cells and the intramural nervous elements, which are widely accepted to be of neural crest origin, in the finch proventriculus may give some support to this postulation of Pearse. However, it should be noticed that Monesi (1960a, b) showed the epithelial origin of enterochromaffin cells by tissue culture, Penttilä (1968) revealed their intraepithelial development by electron microscopy and Kataoka (1969) suggested the entodermal epithelial origin of basal granulated cells by demonstrating an occasional cell containing both endocrine and exocrine granules in the mouse fundic gland resembling acinar-islet cells of the pancreas.

The problem of whether the endocrine cells of the finch proventriculus develop in the epithelium and then migrate into the lamina propria or vice versa is highly interesting in considering the ancestor of the GEP endocrine cells.

シュウシマツ前胃粘膜の神経内分泌複合体に関する電子顕微鏡的研究

片 岡 勝 子

シュウシマツの前胃粘膜は少なくとも2種の内分泌細胞——大型の丸い分泌顆粒をもつⅠ型細胞と、小型でさまざまな電子密度の分泌顆粒や空胞状の顆粒をもつⅡ型細胞
References


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