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Short Communication

Occurrence of different secretin-like cells in the digestive tract of the ascidian *Styela plicata* (Urochordata, Ascidiacea)

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Summary. Secretin-like cells have been detected in the digestive tract of the ascidian *Styela plicata* by means of immunofluorescent and immunocytochemical methods.

Especially, in the esophageal epithelium there are immunoreactive cells (S_2) in which a biogenic amine (5-HT) and a regulatory peptide (secretin) occur together. In the gastric epithelium only secretin-like cells (S_1) are present.

Tests of cross-reactivity performed with glucagon, GIP and VIP, have confirmed the presence of a secretin-like molecule only in the S_1 and S_2 cells.

Key words: Secretin-like cells – Immunocytochemistry – Digestive tract – *Styela plicata*

In recent years investigations of the digestive tract of ascidians have produced extensive evidence of the production of several regulatory peptides, active on the processes of digestion, such as insulin (Falkmer 1967; Davidson et al. 1971), glucagon (Assan et al. 1969), gastrin (Fritsch et al. 1978), and somatostatin (Falkmer et al. 1977, 1978; Fritsch et al. 1978). Recently Bevis and Thorndyke (1979) have localized a secretin-like immunoreactivity in the gastric epithelium of *Styela clava*.

The present work is part of a cytochemical, immunocytochemical and ultrastructural study of polypeptide hormone-producing cells of ascidians, with the aim of verifying and confirming the presence of secretin-like cells throughout the entire digestive tract of an ascidian.

Materials and methods

The specimens of *Styela plicata* (35 mm long) were gathered during April from the water inside the port of Genova. After dissection, esophagus, stomach and intestine were fixed in Bouin in sea water (4 h) or in 10% formaldehyde in sea water (3 h) after which they were dehydrated, embedded in Paraplast and serially sectioned.

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Cytochemistry. Bouin-fixed sections (4 µm) were stained by the following methods: Grimelius silver impregnation (Grimelius 1968), Masson argentaffin reaction, lead-haematoxylin (Solcia et al. 1969), masked metachormasia (Solcia et al. 1968), Gomori aldehyde fuchsin. Formaldehyde-fixed sections (4 µm) were used for the localization of serotonin (5-HT) using the fluorescence method of Sakharova and Sakharov (1968).

Immunofluorescence. The indirect immunofluorescent method (Coons et al. 1955) was applied to Bouin-fixed sections using rabbit antiserum to pure porcine secretin (batch no. R-787502; Milab, Sweden) (diluted 1:2,500 in phosphate buffered saline-PBS) as the first layer and FITC-conjugated goat anti-rabbit γ-globulin (Behring Institut) (diluted 1:100 in PBS) as the second. After incubation for 12–24 h in a moist chamber at 4 °C, sections were washed in PBS following each incubation. Sections were mounted with glycerol-PBS (9:1, v/v) and examined with a Zeiss epifluorescence microscope.

Control staining was carried out with the second layer only, or with anti-secretin preabsorbed overnight at 4 °C with 150 µg/ml diluted antiserum of the following antigens: secretin (lot 101876; Calbiochem-Behring Corp., USA), glucagon (Sigma Chem Co., USA), gastric inhibitory peptide-GIP (Paesel GmbH Co., FRG), vasoactive intestinal peptide-VIP (Prof. V. Mutt, Karolinska Institute, Sweden). Sections of rabbit duodenum were also used as positive controls.

Immunocytochemistry. The peroxidase-antiperoxidase (PAP) method (Sternberger 1974) was applied to Bouin-fixed sections using as a first layer the antiserum anti-secretin (diluted 1:2,500 and applied for 24 h at 4 °C). Following rinsing in PBS for 30 min, the sections were incubated with diluted antiserum to rabbit IgG (raised in goat) for 1 h at room temperature. After rinsing for 30 min in PBS, the sections were incubated for 30 min at room temperature with rabbit-PAP-complex (lot 4-1597; Polysciences, USA) diluted 1:100 in 0.05 M Tris buffer, pH 7.6). Controls were carried out using only the rabbit-PAP-complex and endogenous peroxidase was blocked with 1.2% H_2O_2 in 0.05 M Tris buffer, pH 7.6.

Results

Esophagus. Within the simple columnar epithelium there were argentaffin Masson-positive granulated cells (Fig. 1 b), which were fluorescent with the aqueous formaldehyde method for serotonin (5-HT) (Fig. 1 a).

After incubation with anti-secretin, the serotonin-containing cells were immunofluorescent (Fig. 1 c) and immunostained with the rabbit-PAP-complex (Fig. 1 d). A diffuse weak fluorescence was still detectable after incubation with antiserum preabsorbed with secretin or with FITC-conjugated goat anti-rabbit γ-globulin alone. Using antiserum anti-secretin preabsorbed with glucagon, GIP and VIP respectively, the esophageal secretin-like (S_2) cells were still strongly fluorescent.

Stomach. Immunofluorescent cells (Fig. 1 e) are present only between the ciliated mucous cells of the apices of the gastric folds and extend to the gastric lumen. The same cells were detected by the immunocytochemical method and displayed mainly subnuclear assembled immunoreactive substances (Fig. 1 f). Using anti-secretin preabsorbed with secretin, the gastric secretin-like (S_1) cells could not be detected, whereas incubations with anti-secretin preabsorbed with glucagon, GIP and VIP allowed us to localize these immunoreactive cells which displayed strong fluorescence. The gastric secretin-like (S_1) cells were not restained by the Grimelius and Masson reactions and were negative to lead-haematoxylin, aldehyde fuchsin and masked metachromasia stains.

Intestine. No cells immunoreactive to antiserum for secretin could be seen within the epithelium of the upper and lower intestine. Instead there are few cells per section that were positive to the diazonium reaction and weakly Grimelius positive.

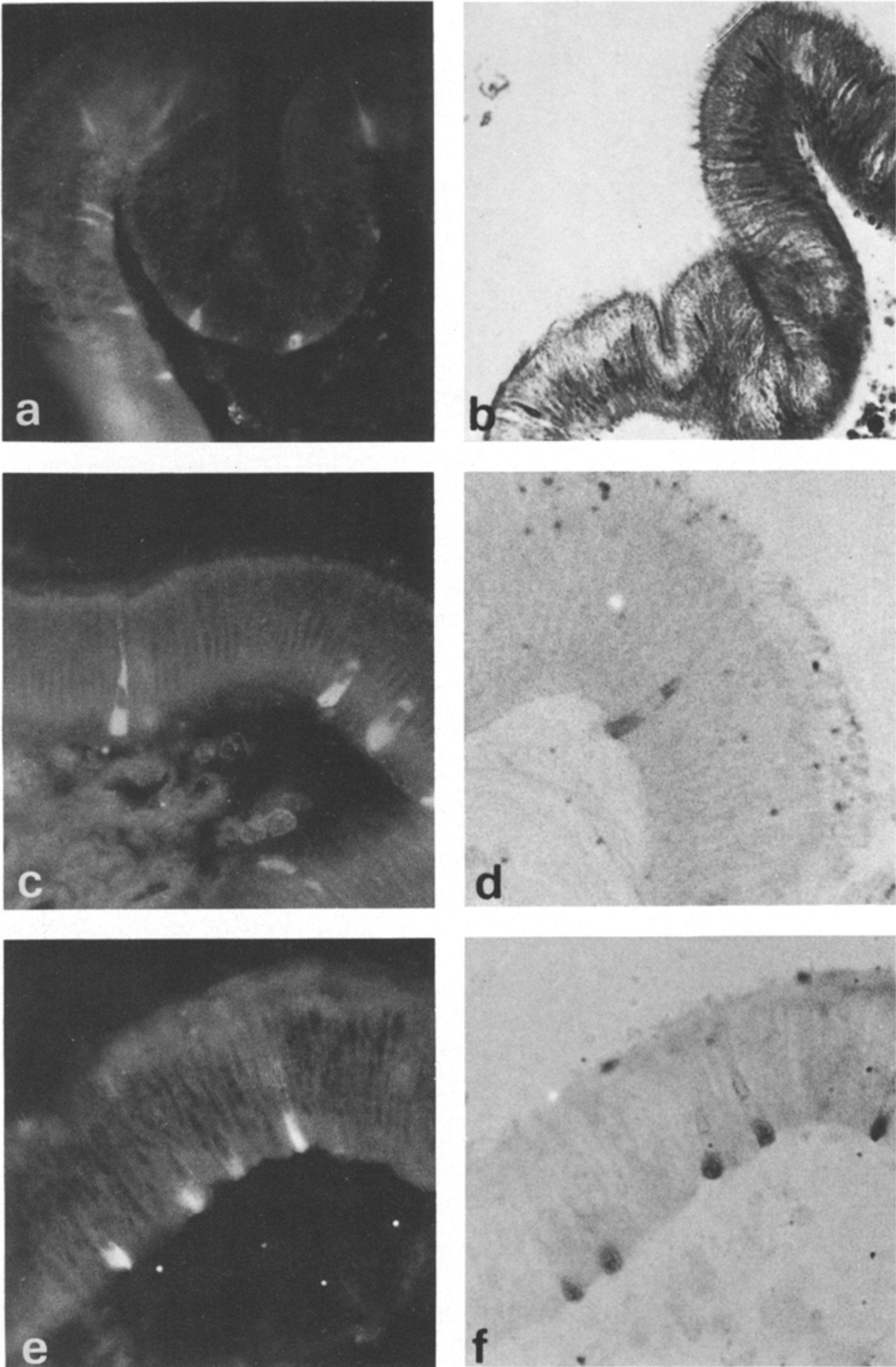

Fig. 1a–f. Esophageal epithelium: **a** fluorescent serotonin-containing cells (×450); **b** argentaffin Masson-positive cells (×450); **c** immunofluorescent (×600), and **d** PAP-immunostained (×700) secretin-like cells. Gastric epithelium: **e** immunofluorescent (×400), and **f** PAP-immunostained (×400) secretin-like cells

Discussion

Secretin cells have been detected by cytochemical, immunocytochemical and ultrastructural methods in several mammals (Bussolati et al. 1971; Solcia et al. 1972; Chey and Escoffery 1976; Larsson et al. 1977). Furthermore a secretin-like factor has been extracted from the intestine of the holocephalian fish *Chymaera monstrosa* (Nilsson 1970) and of the pike *Esox lucius* (Dockray 1974). In protochordates, Bevis and Thorndyke (1979) have found secretin-like immunoreactive cells only in the gastric epithelium.

In the present study, esophageal (S_2) and gastric (S_1) secretin-like cells have been localized in the ascidian *Styela plicata*. The S_1 cell produces a secretin-like factor, while the S_2 cell seems to produce simultaneously a secretin-like factor and serotonin (5-HT). The weak fluorescence visible after incubation with antiserum preabsorbed with secretin is due to the presence of serotonin, which is fluorescent because of its linkage with fluoresceinated serum. The tests of cross-reactivity performed with peptides of the secretin family prove that the S_1 and S_2 cells contain exclusively secretin-like substances.

Both S_1 and S_2 cells have a main basal concentration of secretin-like immunoreactive substances and a positive slender cytoplasmic apex, reaching the lumen. These morphological features lead us to consider these endocrine cells as "open", i.e., they respond directly to luminal stimuli by hormone secretion into the blood stream (Fujita and Kobayashi 1974).

The physiological role of secretin-like factors in ascidians is still unknown. But recently Bevis and Thorndyke (1981) have demonstrated in *Styela clava* that secretin does not produce a significant elevation of levels of protein and acid phosphatase as measured by a perfusion technique.

The immunological affinity between a mammalian antiserum and an ascidian antigen allows us to suggest that the ascidian secretin-like molecule has many antigenic sites in common with mammalian secretin and, therefore, may have a high degree of structural similarity. The presence of a secretin-like molecule in protochordates inclines us to believe that the mechanism of gene duplication followed by subsequent mutations from an ancestral molecule must have appeared before the Mesozoic era, contrary to the postulation of Weinstein (1968). In particular this hypothesis is supported by the presence of secretin-like substances in invertebrates such as cephalopod and gastropod molluscs (Ledrut and Ungar 1936; Van Noorden et al. 1980), which appeared on the earth in the early Paleozoic era.

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