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Distribution, histochemistry and ultrastructure of somatostatin-like immunoreactive cells in the gastroenteric tract of the cartilaginous fish *Scyliorhinus stellaris* (L.)

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Summary

Somatostatin-like immunoreactive cells of an open type have been identified in the digestive tract of the cartilaginous fish *Scyliorhinus stellaris* (L.) by the use of immunocytochemical techniques. In the stomach these cells are numerous both in the corpus (neck zone and tubular glands) and in the pyloric portion (crypts). In the spiral valve, somatostatin-like cells are rare, situated in the intestinal epithelium and without any particular localization. Using semithin serial sections, somatostatin-like cells are found to be Davenport-negative and weakly positive towards the Grimelius silver reaction, and using the semithin and ultrathin technique have been identified at the ultrastructural level; their secretory granules appear electron dense, round or slightly polygonal, and with a limiting membrane tightly adherent to the core. The mean diameter varies from 250–300 nm.

Introduction

Somatostatin has been demonstrated in the digestive tract of different species of bony and cartilaginous fish by both radioimmunoassay and immunocytochemistry (Johnson *et al.*, 1976; Falkmer *et al.*, 1977, 1978, 1981; Klein, 1977; Noaillac-Depeyre & Hollande, 1981; Oyama *et al.*, 1982; El-Salhy, 1984). This neuropeptide seems to be localized not only in endocrine cells but also in nerve fibres of the gut wall (Holmgreen, 1983; Holmgreen & Nilsson, 1983; Reinecke *et al.*, 1983).

In bony fish somatostatin-immunoreactive cells have been identified ultrastructurally by the use of correlative light and electron microscopy (Klein & Van Noorden, 1978; Stefan & Falkmer 1980). Pyloric (Tagliafierro *et al.*, 1984) and pancreatic endocrine cells (Kobayashi & Syed Ali, 1981) have been studied at ultrastructural level in the cartilaginous fish *Scyliorhinus stellaris* and classified tentatively. Pancreatic and pyloric

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presumptive somatostatin-like cells with different granule morphology have been described but no correlations have been made with immunocytochemical techniques.

The aim of the present work is to identify at the electron microscope level the pyloric somatostatin cell in *S. stellaris* by using the reliable semithin and ultrathin technique to correlate immunocytochemical and ultrastructural data. In addition we report the distribution, not yet examined, of this endocrine cell type along the gastrointestinal tract of the same cartilaginous fish.

Materials and methods

Specimens of *Scyliorhinus stellaris* (L.), caught in the gulf of Genoa, were killed with Sandoz MS 222 (1% in sea water).

For immunofluorescence and immunoperoxidase studies, samples of stomach (corpus and pyloric portion) and spiral valve were fixed in formalin–calcium (1% $CaCl_2$ in 4% formaldehyde) and embedded in Paraplast (Sherwood Medical, USA). Dewaxed sections, 4 μm thick, were exposed to somatostatin antiserum, raised against synthetic ovine cyclic somatostatin conjugated to thyroglobulin (Milab, Sweden) and diluted 1:1600 in 0.1 M phosphate-buffered saline (PBS), pH 7.2, in a moist chamber for 24 h at room temperature (Coons *et al.*, 1955). Following a rinse in PBS for 30 min, the sections were incubated in a moist chamber for 1 h at room temperature with goat anti-rabbit γ-globulin conjugated with fluorescein isothiocyanate (Berhing Institute) and diluted 1:100 in PBS. The sections were rinsed for 30 min and examined with a Zeiss epifluorescence microscope. Subsequently the sections were treated with the peroxidase–antiperoxidase (PAP) method of Sternberger (1979) using the rabbit PAP complex (UCB, Brussels) diluted 1:100 in PBS in a moist chamber for 1 h at room temperature and stained by a fresh solution of 3, 3'-diaminobenzidine 4 HCl (DAB) (60 mg/100 ml) and hydrogen peroxide (0.01%) in PBS for 1 h. The endogenous peroxidase was previously blocked with 1–2% hydrogen peroxide in PBS. Then the sections were dehydrated, mounted in DPX and examined through an ordinary light microscope. Controls were performed by replacing the specific antiserum with (a) normal rabbit serum and (b) antiserum preabsorbed overnight at 4° C with synthetic somatostatin (Sigma, USA).

For electron microscopy, histochemistry and immunocytochemistry, pieces of pyloric mucosa were fixed in Karnovsky (1965), postfixed in 1% osmium tetroxide, stained with uranyl acetate and embedded in Epon–Araldite.

For correlative histochemical study a series of four adjacent semithin sections were utilized. The resin was removed with sodium ethoxide 3% in absolute alcohol (Canese & Bussolati, 1977). Osmium deposits were removed with 1% periodic acid (Beauvillain *et al.*, 1975). The sections were stained with the following reactions: Masson (Lison, 1960), Grimelius (1968), Davenport (Hellmann & Hellerstrom, 1960) and the indirect immunofluorescence method (Coons *et al.*, 1955). Identification of the immunoreactive cells at the ultrastructural level was made by the consecutive semithin and ultrathin section technique (Canese & Bussolati, 1977). The semithin sections, etched and oxidated as described above, were processed for immunohistochemical demonstration of somatostatin. Adjacent ultrathin sections were placed on a grid, contrasted with lead citrate and examined in a Siemens Elmiskope I electron microscope.

Results

Distribution and morphology

Somatostatin-like immunoreactive cells were observed along the whole gastrointestinal tract. They are more numerous in the pyloric portion than in the corpus of the stomach and rare in the spiral valve. In the mucosa of the corpus (gastric mucosa) they are localized

mostly at the bottom of the gastric pits (neck zone) together with undifferentiated cells, often undergoing mitosis, and other endocrine cell types. A few of them are also present in the gastric glands especially in their upper part (Fig. 1). In the pyloric portion (pyloric mucosa) somatostatin-like cells occur at the base of the crypts together with other endocrine cell types (Fig. 2). In the spiral valve they are scattered among the absorptive intestinal cells (Fig. 3). In the gastric neck zone and in the intestinal epithelium the somatostatin-like cell presents an apical process that is in contact with the lumen. In the gastric and pyloric glands, the cells are still in contact with the lumen but more compact in shape; furthermore some of them possess at the base a short cytoplasmic process which is situated beneath the adjacent cells. No somatostatin immunoreactive fibres are found in any layer of the gastroenteric tract.

Histochemistry

Using semithin serial sections of the pyloric mucosa, somatostatin-like cells are found to be weakly argyrophilic to the Grimelius reaction and negative to the Davenport (HH) reaction (Fig. 4a,b). Many HH positive cells are present in the pyloric crypts but they do not correspond to the immunoreactive cells.

Ultrastructure

The ultrastructure of the pyloric somatostatin-like cells has been defined using the consecutive semithin and ultrathin section technique. Two semithin sections have been used for immunofluorescent and immunoperoxidase reactions and the ultrathin one for electron microscopy (Fig. 5a–c). The cells showing immunoreactivity at the light microscope level display, at the electron microscope level, round or slightly polygonal granules with a limiting membrane tightly adherent to the core. The matrix shows a fine granulated texture, quite electron dense (Fig. 6). A few granules show a central cavitation. The mean diameter varies from 250–300 nm. They are distributed throughout the cell except in the Golgi zone. In the basal part of the cell the granules display a characteristic arrangement in rows. The nucleus often is irregular in shape and has a well developed nucleolus.

Discussion

This study demonstrates that somatostatin-like immunoreactive cells are distributed along the length of the *S. stellaris* gastrointestinal tract. Their frequency correlates well with previous data about the concentration of somatostatin-like immunoreactivity, obtained both by radioimmunoassay (Falkmer *et al.*, 1977) and immunocytochemistry (El-Salhy, 1984) in other selachians and by immunocytochemistry in teleosts (Noaillac-Depeyre & Hollande, 1981; Holmgreen *et al.*, 1982).

The distribution of the gastric somatostatin-like cells differs from that found in bony fish (Noaillac-Depeyre & Hollande, 1981; Reinecke *et al.*, 1983) being more similar to that of mammals: in effect these cells are located in the upper part of the gastric gland near

hydrochloric acid-secreting cells (our data, not yet published); in the same zone it is also possible to find immunoreactive cholecystokinin-like cells (Tagliafierro & Faraldi, 1984). The topographical arrangement, together with the physiological data about cholecystokinin (Vigna, 1983) and somatostatin-like peptides (Konturek, 1980; Hakanson *et al.*, 1981), supports the hypothesis that the regulative pattern of the gastric secretion in selachians is similar to that found in higher vertebrates.

Holmgreen & Nilsson (1983) detected somatostatin immunoreactive nerves in the *Squalus acanthias* gut wall but we were unable to find them. This discrepancy could be related to different fixatives, technical procedures and antibodies used. As in bony fish (Ezeasor, 1981) and protochordates (Pestarino, 1983) all somatostatin-like cells are in contact with the lumen, even in the gastric portion, and generally have cytoplasmic processes which are relatively short in *S. stellaris*.

The silver reaction of Hellmann–Hellerstrom, even if not fully understood, has long been the most frequently used method for identifying the somatostatin cells in mammals (Canese & Bussolati, 1974; Solcia *et al.*, 1980). Nevertheless, this method is not always useful in lower vertebrates and protochordates. In effect in cyclostomes (Van Noorden *et al.*, 1977) and protochordates (Fritsch *et al.*, 1978; Pestarino, 1983) somatostatin-like cells are found to be HH negative. In some teleosts they are HH positive (Klein & Van Noorden, 1978), in others HH negative (Wagner & McKeown, 1981). D cells, HH positive, were previously found in the pancreas of some cartilaginous fish (Falkmer *et al.*, 1977, Kobayashi & Syed Ali, 1981). Also in the gastrointestinal tract of *S. stellaris* we have found HH positive cells but they never correspond to the somatostatin-like immunoreactive ones. If it is actually the somatostatin itself making the somatostatin-cells of mammals HH positive, then the somatostatin-like substance of protochordates and lower vertebrates differs in some part of its molecular structure, not correlated to the immunoreactive sequence, with that found in mammals. In effect the somatostatin

Fig. 1. Gastric mucosa. Arrows indicate the immunofluorescent somatostatin-like cells in the neck zone and in the tubular glands. × 500.

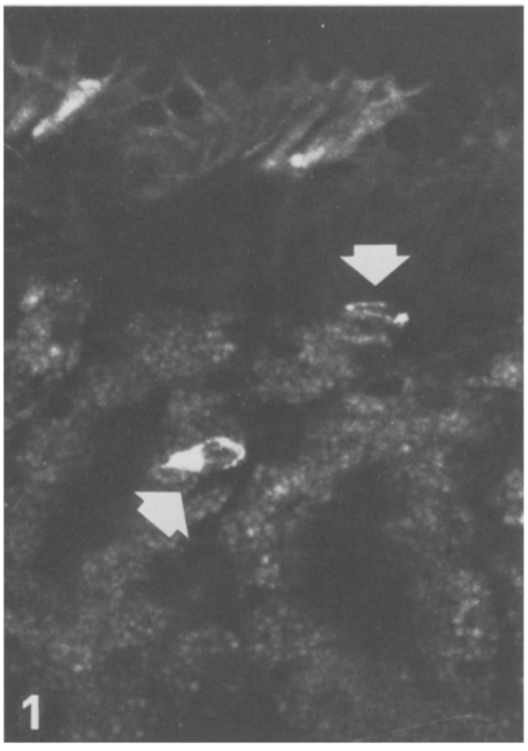
Fig. 2. Pyloric mucosa. The immunofluorescent somatostatin-like cells are localized at the bottom of pyloric crypts. × 500.

Fig. 3. Intestinal mucosa. Two somatostatin-like cells (PAP staining only) in the intestinal epithelium. × 500.

Fig. 4. Pyloric mucosa. Adjacent semithin sections (Karnovsky fixed and resin embedded). (a) The immunofluorescent somatostatin-like cell (arrows). (b) is HH negative (arrows). (a) Immunostaining with antisomatostatin antiserum. × 500. (b) Silver reaction according to Hellman & Hellerstrom. V, blood vessel. × 500.

Fig. 5. Pyloric crypt. Correlative light and electron microscopy. (a) and (b) are consecutive semithin sections. Immunofluorescent (a) and immunoperoxidase positive (b) somatostatin-like cell. (c) Adjacent ultrathin section. The same cell as shown in (a) and (b), exhibits numerous secretory granules. (a) × 2500. (b) × 2500. (c) × 12 500.

Fig. 6. Somatostatin-like cell. Detail of the secretory granules. × 35 000.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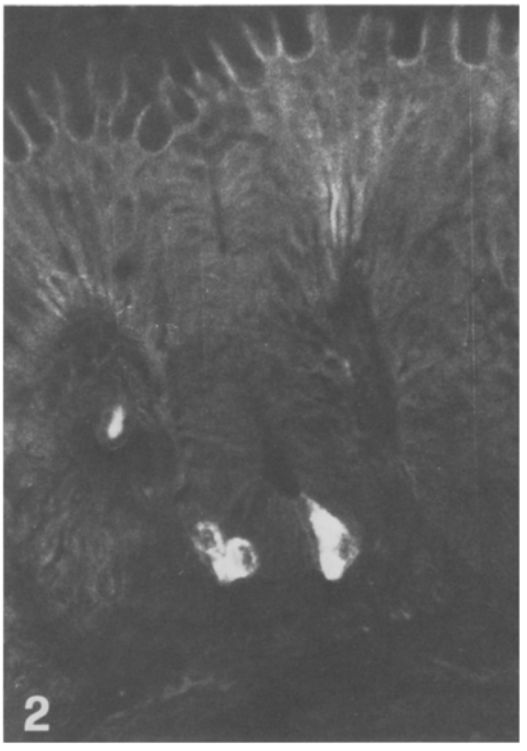
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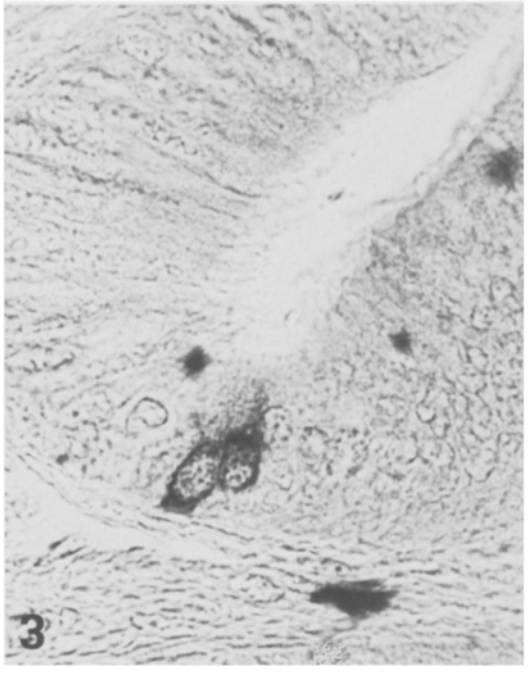
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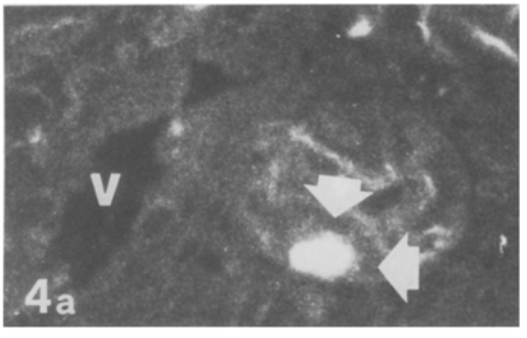
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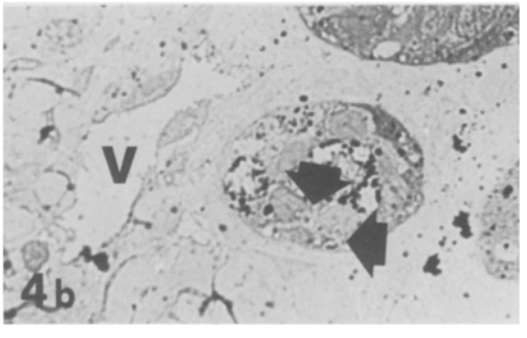
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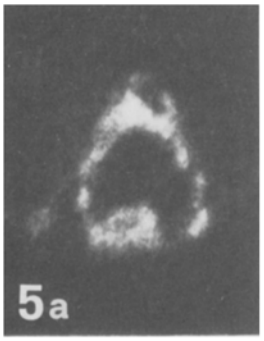

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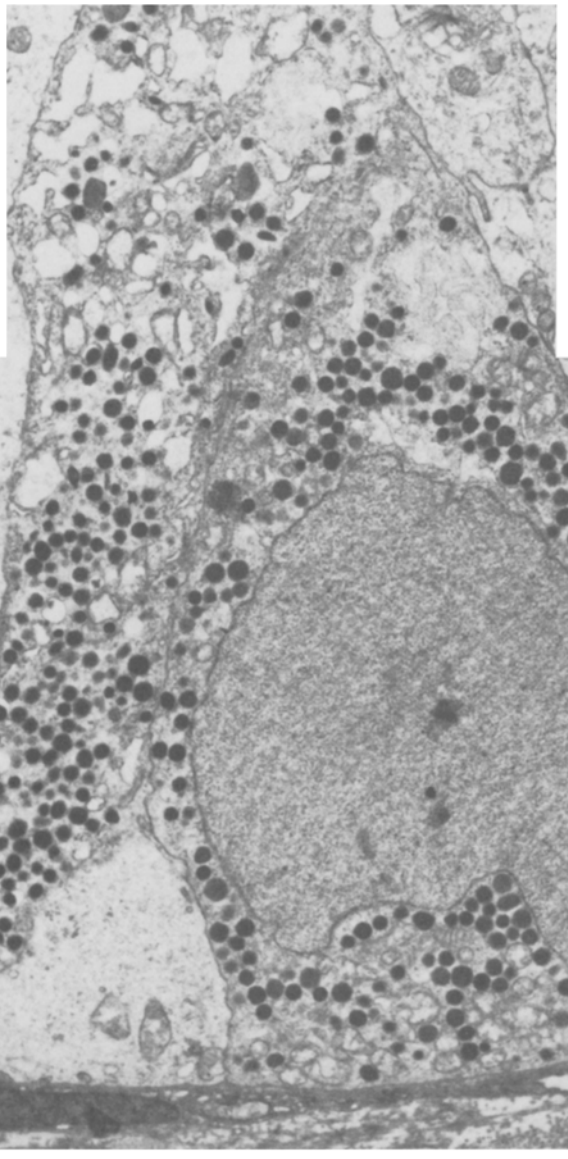

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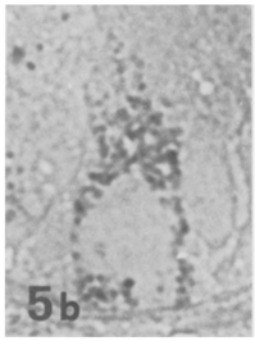

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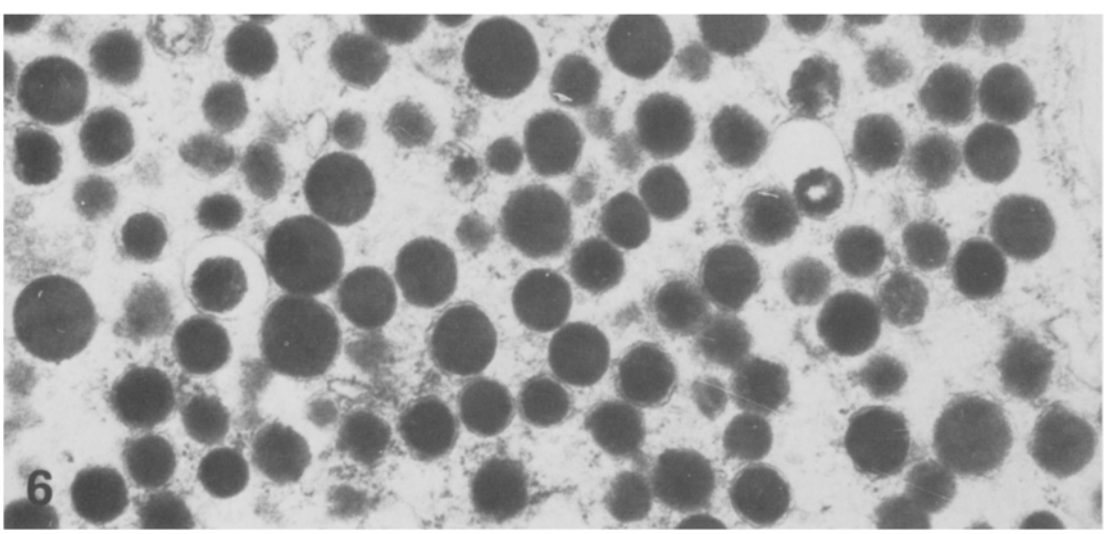

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substance extracted from the catfish (Oyama *et al.*, 1982) has a different structure on its *N*-terminal, being larger and more acidic; such modification does not affect its biological activity but could influence its histochemical characteristics.

At the electron microscope level, Kobayashi & Syed Ali (1981) describe in the pancreas of *S. stellaris* a probable D cell whose granule characteristics are partly similar to those of the pyloric somatostatin-like immunoreactive cells here identified with the semithin and ultrathin technique. On the contrary the granules of pyloric somatostatin-like cells of *S. stellaris* are strictly similar to those of many other species, for example to those of catfish pancreatic somatostatin cells (Oyama *et al.*, 1982) or to those of cat, man and chicken pyloric somatostatin ones (Alumets *et al.*, 1977; Lehy *et al.*, 1981). Considerable differences have instead been noted between the granules of pyloric somatostatin-like cells of *S. stellaris* and those of some other bony fish (Klein & Van Noorden, 1978; Stefan & Falkmer, 1980). As already emphasized elsewhere (Klein, 1977) we think that the different appearance of the granules observed in these cells depends more on the use of a particular fixative than on the different species studied. In effect every time we noticed different granule morphology, different fixatives had been used; while similar granule morphology corresponded with the use of the same or similar fixative. For this reason we think that correlation between different species could only be done usefully if the same fixative is employed.

Immunohistochemical techniques correlated with ultrastructural ones appear to be the only useful approach by which to solve the problem of identification of endocrine cells.

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