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Use of Immunocytochemical Staining of Somatostatin for Correlative Light and Electron Microscopic Investigation of D Cells in the Pancreatic Islet of *Xiphophorus helleri* H. (Teleostei)*

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Summary. Somatostatin-containing cells have been demonstrated by immunocytochemistry in semithin sections of the pancreatic islet of the teleost fish, *Xiphophorus helleri*. These cells were shown by correlative light and electron microscopy to be identical with D cells previously defined in this species by the silver impregnation method of Hellman and Hellerström.

Key words: Pancreatic islets – Correlative light and electron microscopy – Immunostaining – Somatostatin – *Xiphophorus helleri*.

Resumé. – L'étude en immunofluorescence de tissu fixé et inclus pour la microscopie électronique a démontré l'existence de somatostatine dans le pancréas endocrine du téléostéen *Xiphophorus helleri*. L'observation comparée de coupes sériées ultrafines et sémifines a permis d'établir l'identité entre les cellules positives et les cellules qui avaient été précédemment définies comme cellules D, en utilisant l'imprégnation argentique de Hellman et Hellerström.

Somatostatin has been clearly localised in the pancreatic D cells of many mammals; observations on different species of fishes are, however, still rare. Somatostatin has been detected by radioimmunoassay in the pancreatic tissue of the dogfish, *Squalus acanthias*, and the principal islets of a teleost fish, *Cottus scorpius* (Falkmer et al., 1977). Immunohistochemical staining has been used to demonstrate somatostatin in the islets of *Ictalurus punctata* and *Lophius americanus* (Johnson et al., 1976) and in *Cottus scorpius* (Falkmer et al., 1977), and was reported in various other species of

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fishes (Van Noorden and Patent, 1978). In all these studies, paraffin-embedded material was used and no correlation was made with a definite ultrastructural cell type.

In *Xiphophorus helleri,* D cells were described by means of correlative light and electron microscopy (Klein, 1975; Klein and Lange, 1977). The D cells were identified by light microscopy in semithin resin-embedded sections using the silver impregnation technique of Hellman and Hellerström (1960). The small size of the D cell granules in comparison with the granules of the other islet cell types seemed to be a constant characteristic. We have now extended and confirmed these findings by using the semithin-ultrathin technique to correlate immunofluorescence for somatostatin with electron microscopy.

Materials and Methods

I. Preparation of the Sections

In *Xiphophorus helleri* a single islet contains all the endocrine pancreatic tissue. Adult males and females were killed by decapitation. Islets were removed and fixed in a variety of solutions including glutaraldehyde, glutaraldehyde with formaldehyde, and formaldehyde. Preliminary experiments showed that the most satisfactory fixative for preservation of the immunoreaction was methanol-free formaldehyde (MFF) at a concentration of 2% or 4% in 0.1 M phosphate buffer, pH 7.4, containing 0.1 M sucrose (Polak et al., 1971). Fixation was carried out for 2 h at room temperature. The material was embedded in Araldite. Serial semithin (500 nm to 1000 nm) and ultrathin sections were cut. The ultrathin sections were collected on central slot grids which provided an uninterrupted viewing range of

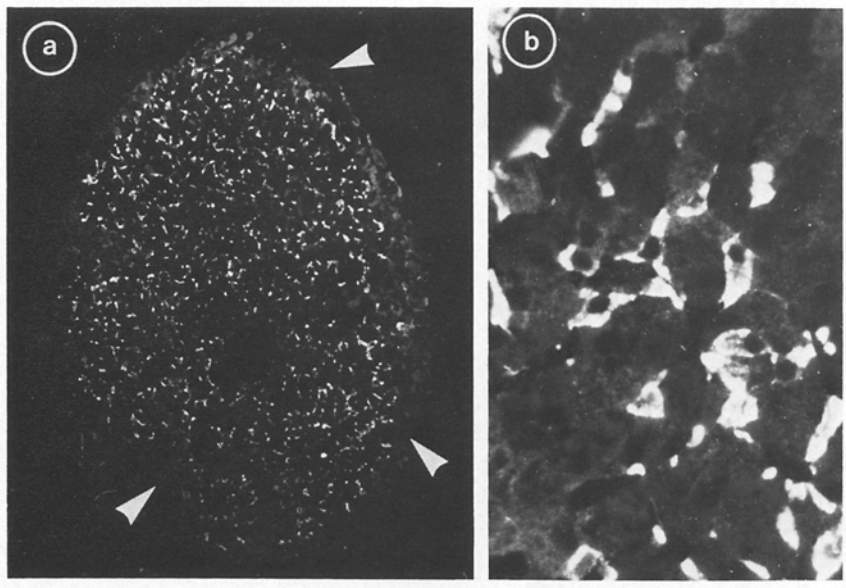

Fig. 1 a and b. Somatostatin-secreting cells in the pancreatic islet of *Xiphophorus helleri* (2% MFF in phosphate buffer, 2 h). Indirect immunofluorescence with rabbit anti-somatostatin serum and fluorescein-labelled goat anti-rabbit globulin serum. **a** View of the entire islet. Positive cells are scattered throughout the islet, except for the peripheral area (*arrows*). × 150. **b** Positive cells, detail. The cytoplasm is strongly fluorescent; nuclei appear black. Note the triangular shape of the cells. × 800

2 mm × 1 mm. This facilitated finding the cells which had been previously located on the adjacent semithin section.

II. Immunostaining

Semithin sections were mounted on glass slides. The resin was removed with a saturated solution of sodium hydroxide in absolute ethanol (Lane and Europa, 1965). The indirect immunofluorescence technique was then applied (Coons et al., 1955).

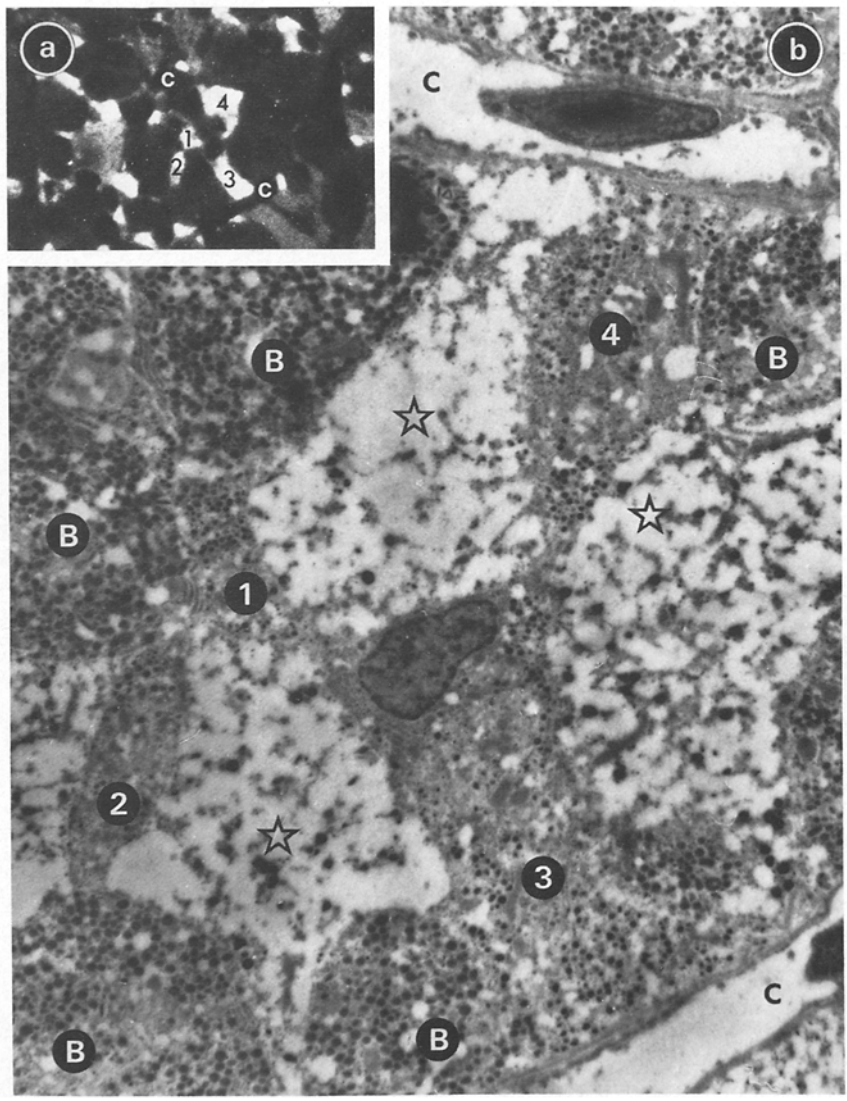

Fig. 2 a and b. D cells, correlative light and electron microscopy (2% MFF in phosphate buffer, 2 h, **a** Semithin section, specific incubation with anti-somatostatin serum observed in UV light. The numbers refer to cells identified in the electron microscope (Fig. 2b). × 800. **b** Adjacent ultrathin section. × 7700. Positive D cells (*1–4*) exhibit secretory granules much smaller than those of other islet cells. *B*, B cells; *c*, capillary; "clear cells" (☆) are badly preserved with this fixative

1) Specific Incubation. First layer (serum I): rabbit antibody to synthetic somatostatin at a dilution of 1/2,400 for 18 to 24 h at 4° C. Second layer (serum II): goat antibody to rabbit globulin labelled with fluorescein isothiocyanate at a dilution of 1/10 for 1 h at room temperature.

2) Controls. a) Serum I absorbed with somatostatin (4 nMoles/ml of diluted antiserum) followed by serum II. b) Normal rabbit serum followed by serum II. c) Serum II alone. d) No serum, control for primary or formaldehyde-induced fluorescence.

III. Correlative Study

Comparison of the semithin with their adjacent ultrathin sections allowed the immunostained cells to be identifed in the electron microscope.

Results

Many cells were immunofluorescent after incubation with the anti-somatostatin serum. They were distributed throughout the islet except for the peripheral area (Fig. 1a). The cells were usually narrow and often triangular in shape (Fig. 2b). All the staining controls were negative.

Correlation of the light and electron microscopical observations (Fig. 2) showed that the somatostatin-positive cells were in fact the cells which were previously described as D cells. The cell granules were round and electron dense and were always strikingly smaller than the granules of other islet cells. The MFF fixative preserved both D and B cell granules quite well, but seemed to be a very poor fixative with regard to the "clear cells" (Fig. 2b).

Discussion

The silver impregnation technique of Hellman and Hellerström (1960) has long been the most frequently used method of identifying D cells, although the mechanism of the reaction is still not understood. The product of the cells which it stained was not known until 1975 when somatostatin was demonstrated in the D cells of mammalian islets (Hökfelt et al., 1975; Orci et al., 1975; Pelletier et al., 1975; Polak et al., 1975). Since then, many investigations have been carried out using immunocytochemical staining with antiserum to somatostatin, but there have been very few studies in fishes. It has now been shown that antibodies to synthetic somatostatin stain certain cells in fish islets and positive results have been reported in various species (Johnson et al., 1976; Falkmer et al., 1977; Van Noorden and Patent, 1978). In all these investigations Bouin-fixed, paraffin-embedded material was used.

We have now been able to correlate the light and electron microscopical appearance of somatostatin-containing cells in one teleost species, *Xiphophorus helleri*, by specific immunostaining for somatostatin on semithin resin-embedded sections compared with their adjacent ultrathin sections. In this species D cells have already been identified by the Hellman and Hellerström silver impregnation on semithin sections, and their ultrastructural characteristics established by comparison with adjacent ultrathin sections (Klein, 1975; Klein and Lange, 1977). The

cells which we have now shown to contain somatostatin are identical with the D cells previously described and show the same highly electron dense, round granules, smaller than those of other islet cell types. Although the size of secretory granules cannot be used as an absolute criterion in defining a cell type, it should be borne in mind that in the islet of *Xiphophorus helleri* D cells were the only cell type possessing such small granules and this feature was constant, regardless of the fixative used (Klein, 1975; Klein and Lange, 1977). The formalin fixation which was used in this investigation was obviously inadequate for some cell types (e.g. the "clear cells", Fig. 2b). Moreover, fixation which preserves peptide antigenicity is usually sub-optimal for electron microscopic morphology, so that the good preservation of the D cells in this case was fortuitous. However, D cells have already been shown to be well preserved by a variety of fixation procedures (Klein, 1975; Klein and Lange, 1977).

The ultrastructural appearance of granules defined as those of D cells varies considerably according to species. Among the teleosts the only descriptions of D granules corresponding to those of *Xiphophorus helleri* are given for *Cyprinus carpio* by Faller and Lange (1969) and by Nakamura and Yokote (1971). The general lack of correlative light and electron microscopy makes discussion of this point extremely difficult, particularly as this type of investigation (with Hellman and Hellerström silver impregnation on semithin sections) has been carried out in only two species, *Xiphophorus helleri* (Klein, 1975; Klein and Lange, 1977) and *Fugu rubripes* (Kobayashi et al., 1976). The D cell granules of the latter species were larger and less electron dense than those of *Xiphophorus helleri*. Teleost islet cells have frequently been assumed to be D cells because of their ultrastructural resemblance to mammalian D cells with large granules of low electron density. Based on the present study of *Xiphophorus helleri*, this assumption may not be valid. It has been emphasised elsewhere (Klein, 1977) that it is difficult to define D cells of teleosts using ultrastructural features alone and that correlative light and electron microscopy should be used in order to avoid misinterpretation. In this respect the use of immunocytochemical staining for somatostatin for identification of D cells appears to be particularly promising.

References

Coons, A.H., Leduc, E.H., Connolly, J.M.: Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. J. exp. Med. **102,** 49–59 (1955)

Falkmer, S., Elde, R.P., Hellerström, C., Petersson, B., Efendić, S., Fohlman, J., Siljevall, J.B.: Some phylogenetical aspects on the occurrence of somatostatin in the gastro-entero-pancreatic endocrine system. A histological and immunocytochemical study, combined with quantitative radioimmunological assays of tissue extracts. Arch. histol. jap. **40** (suppl.), 99–117 (1977)

Faller, A., Lange, R.H.: Kombinierte licht- und elektronenmikroskopische Analyse des Zellbildes in den Langerhansschen Inseln des Karpfens. Arch. histol. jap. **31,** 73–80 (1969)

Hellman, B., Hellerström, C.: The islets of Langerhans in ducks and chickens with special reference to the argyrophil reaction. Z. Zellforsch. **52,** 278–290 (1960)

Hökfelt, T., Efendić, S., Hellerström, C., Johansson, O., Luft, R., Arimura, A.: Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A1-cells of the pancreatic islets and to the hypothalamus. Acta endocr. (Kbh.), Suppl. **200,** 1–41 (1975)

Johnson, D.E., Torrence, J.L., Elde, R.P., Bauer, G.E., Noe, B.D., Fletcher, D.J.: Immunohistochemical localization of somatostatin, insulin and glucagon in the principal islets of the anglerfish (*Lophius americanus*) and the channel catfish (*Ictalurus punctata*). Amer. J. Anat. **147,** 119–124 (1976)

Klein, C.: Etude ultrastructurale et cytochimique du pancréas endocrine d'un Poisson téléostéen, *Xiphophorus helleri* H. Thèse de Doctorat d'Etat (n° 909) Strasbourg, Université L. Pasteur, n° CNRS AO 11.678, 1975

Klein, C.: Ultrastructural and cytochemical bases for the identification of cell types in the endocrine pancreas of teleosts. Intern. Rev. Cytol., Suppl. **6,** 289–346 (1977)

Klein, C., Lange, R.H.: Principal cell types in the pancreatic islet of a teleost fish, *Xiphophorus helleri* H. Cell Tiss. Res. **176,** 529–551 (1977)

Kobayashi, K., Shibasaki, S., Takahashi, Y.: Light and electron microscopic study on the endocrine cells of the pancreas in a marine teleost, *Fugu rubripes rubripes*. Cell Tiss. Res. **174,** 161–182 (1976)

Lane, B.P., Europa, D.L.: Differential staining of ultrathin sections of epon-embedded tissues for light microscopy. J. Histochem. Cytochem. **13,** 579–582 (1965)

Nakamura, M., Yokote, M.: Ultrastructural studies on the islets of Langerhans of the carp. Z. Anat. Entwickl.-Gesch. **134,** 61–72 (1971)

Orci, L., Baetens, D., Dubois, M.P., Rufener, C.: Evidence for the D-cell of the pancreas secreting somatostatin. Horm. metab. Res. **7,** 400–402 (1975)

Pelletier, G., Leclerc, R., Arimura, A., Schally, A.V.: Immunohistochemical localization of somatostatin in the rat pancreas. J. Histochem. Cytochem. **23,** 699–701 (1975)

Polak, J.M., Bussolati, G., Pearse, A.G.E.: Cytochemical, immunofluorescence and ultrastructural investigations on the antral G cells in hyperparathyroidism. Virchows Arch. Abt. B Zellpath. **9,** 187–197 (1971)

Polak, J.M., Pearse, A.G.E., Grimelius, L., Bloom, S.R., Arimura, A.: Growth-hormone release-inhibiting hormone in gastrointestinal and pancreatic D-cells. Lancet **1975 I,** 1220–1222

Van Noorden, S., Patent, G.J.: Localization of pancreatic polypeptide (PP)-like immunoreactivity in the pancreatic islets of some teleost fishes. Cell Tiss. Res. **188,** 521–525 (1978)

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