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Short Communication

Localization of Somatostatin-like Immunoreactivity in the Pancreatic Islets of the Hagfish, *Myxine glutinosa* and the Lamprey *Lampetra fluviatilis*

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Summary. Somatostatin-like immunofluorescence has been found by immunostaining in cells of the bile duct mucosa and pancreatic islet parenchyma of the hagfish, *Myxine glutinosa*, and the islet lobules of the lamprey, *Lampetra fluviatilis*.

Key words: Pancreatic islets – Cyclostomes – Somatostatin – Endocrine cells – Immunofluorescence.

Introduction

The mammalian hypothalamic peptide, somatostatin (somatotropin-release inhibiting factor, SRIF), has recently been demonstrated by immunostaining in the D cells of the gut and pancreas where it is also found by extraction and assay methods (Polak et al., 1975). SRIF inhibits the release of many hormones and may have a local action on the hormone producing cells with which the D cells are associated.

Cross reactions occur between antibodies to some mammalian hormones and certain cells in the intestine and pancreatic islets of cyclostomes (Van Noorden and Pearse, 1974; Östberg et al., 1975, 1976a, b). Insulin immunoreactivity, but not that of other polypeptide hormones, has been localised to granulated cells of hagfish and lamprey islets and to scattered cells in the hagfish bile duct epithelium (cf. Östberg, 1976). A second type of endocrine cell with spherical secretion granules has been found in the hagfish islet parenchyma and bile duct mucosa (Thomas et al., 1973; Östberg et al., 1976a). Only one type of granulated cell has been described in lamprey islets, but some of the islet lobules are not composed of typical B cells and are negative for insulin staining (Van Noorden and Pearse, 1974).

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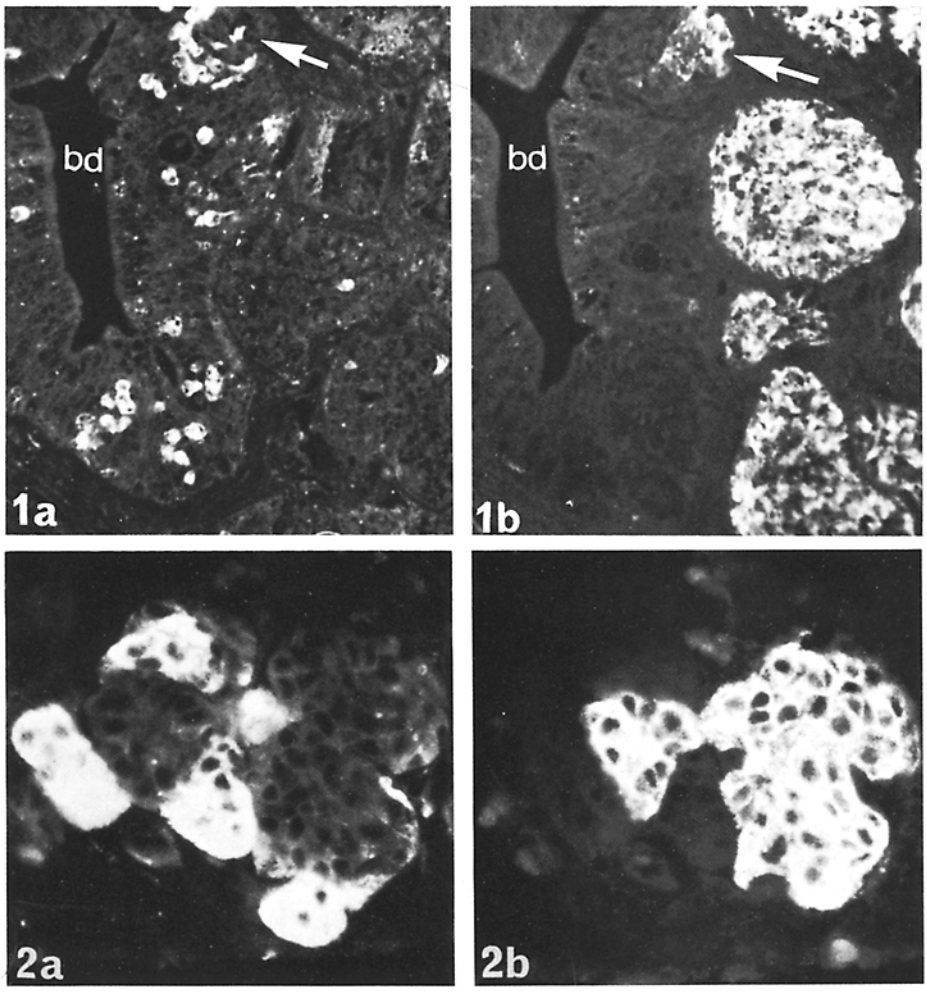

Fig. 1 a and b. Immunofluorescence photomicrographs showing portions of the same hagfish (*Myxine glutinosa*) islet organ. SRIF-like immunoreactivity **a** is seen in cells of the bile duct (*bd*) mucosa and the periphery of a pancreatic islet bud (arrow) in close association with the bile duct epithelium. Some isolated cells are also present in the surrounding islet lobules (right). In a near adjacent section **b** insulin immunoreactive cells are seen in other cells of the islet bud (arrow) and islet lobules. MFF-fixation. × 185

Fig. 2 a and b. Immunofluorescence photomicrographs of areas of the same lamprey (*Lampetra fluviatilis*) islet lobules. SRIF-like immunoreactive cells **a** are seen arranged into separate lobules. In a near adjacent section **b** insulin immunoreactive cells are seen in other lobules of the islet parenchyma. Freeze-dried formaldehyde vapour fixation. × 344

It therefore seemed pertinent, when a sensitive antiserum to SRIF became available, to test the cyclostome islet parenchyma for a possible cross-reaction.

Materials and Methods

Specimens of *Myxine glutinosa* were obtained from the Gullmar fiord, Sweden, and larval and anadromous adult *Lampetra fluviatilis* from the rivers Severn and Trent, England.

Islet tissue was dissected out and fixed in various ways including methanol-free formaldehyde (MFF) (Polak et al., 1971), Bouin's fluid, and freeze-drying followed by vapour fixation in formaldehyde, diethylpyrocarbonate or benzoquinone (Pearse and Polak, 1975).

Antibodies to synthetic cyclic SRIF were produced in rabbits and thoroughly tested by radioimmunoassay and by immunostaining of mammalian SRIF cells (Polak et al., 1975).

The indirect immunofluorescence technique (Coons et al., 1955) was applied to sections using rabbit anti-SRIF as the first layer and fluorescein-conjugated goat anti-rabbit globulin as the second layer. Control staining included the use of non-immune rabbit serum, anti-SRIF which had been absorbed with SRIF, and anti-SRIF which had been absorbed with glucagon, insulin or bovine albumin. Sections adjacent to those stained for SRIF were stained with antisera to mammalian glucagon or to mammalian or teleost insulin. Sections were mounted in buffered glycerine and examined in a Zeiss fluorescence microscope.

Results

In *Myxine* all MFF-fixed islet tissue examined contained large and apparently granular cells immunoreactive with antisera to SRIF. The cells were found sparsely scattered in both solid and follicular islets, singly or in groups, and rather more frequently in the bile duct epithelium. The number of SRIF cells in the islet lobules varied considerably from animal to animal, but where bile duct mucosa was present in the section its epithelium always contained immunoreactive cells (Fig. 1a). Occasionally, SRIF-immunoreactive cells appeared to be part of a newly formed islet bud (Östberg et al., 1976a).

Anti-SRIF serum which had been absorbed with glucagon, insulin or albumin gave positive staining. No staining was seen with the anti-SRIF serum which had been absorbed with SRIF. Antisera to both mammalian and tuna-fish insulin stained the majority of the islet cells and a few cells in the bile duct, but comparison of adjacent sections stained with anti-SRIF and anti-insulin demonstrated that different cells were stained (Fig. 1b).

In the lamprey anti-SRIF sera stained adult islet tissue after various fixations. The islet lobules stained were distinct from the lobules stained by anti-insulin sera (Fig. 2a, b). No staining of islet tissue was seen with anti-glucagon, non-immune sera or anti-SRIF absorbed with SRIF.

No SRIF immunofluorescence was seen in the newly-formed islets of 13 cm larval lampreys, although insulin was intensely immunoreactive.

Discussion

Typical D cells with large (350 nm) spherical granules, staining with the silver impregnation technique of Hellerström and Hellman (1960), have been found not

only in mammals but also in many fishes (Patent and Epple, 1967; Brinn, 1973; Thomas, 1975). They have not yet been reported in cyclostomes, but Epple and Brinn (1975) found four different cell types distinct from the B cell in the islet organ of the sea lamprey, *Petromyzon marinus*, of which one may well turn out to be a D-like cell. No unequivocal Hellman/Hellerström silver-stained cells have been found in cyclostomes.

The finding of SRIF-like immunoreactivity in the *Myxine* islets agrees well with earlier work which showed the presence of an occasional second granular cell type in the islets and bile duct epithelium (Thomas et al., 1973; Östberg et al., 1976a). In addition, experiments on amine precursor uptake, combined with insulin immunofluorescence, showed bile duct APUD cells (Pearse, 1968) which were insulin-negative (Östberg et al., 1975, 1976a). There now seems little doubt that these cells correspond to those cells with spherical secretion granules and that the majority of these cells show SRIF-like immunoreactivity.

In the lamprey pancreas the SRIF-positivity, which is in cells and lobules distinctly separate from those containing insulin, presumably corresponds to the aldehyde fuchsin-negative areas of the pancreas. Winbladh (1966) suggested that these areas, which in her preparations contained empty vesicles, might be the equivalent of the D cells of other chordates. Ermisch (1966) found aldehyde fuchsin-negative granulated cells which are probably the same as the vesiculated cells. The situation will be clarified by reinvestigation of the lamprey islets by a combination of electron microscopy and immunocytochemistry. The stage of development and morphological source of the SRIF cells in lampreys awaits further research as does the physiological role of this hormone-like substance in cyclostomes. It may have hormone-release inhibiting properties and take part in the regulation of insulin secretion, as it does in mammals and probably birds, since the insulin-release inhibiting properties of a pigeon D cell extract were demonstrated several years before the discovery of SRIF (Hellman and Lernmark, 1969). On the other hand, the immunoreactive region may merely be part of a larger peptide belonging to the secretin-glucagon family of hormones which share a four peptide sequence with somatostatin.

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