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Localization of Pancreatic Polypeptide (PP)-like Immunoreactivity in the Pancreatic Islets of Some Teleost Fishes

Susan Van Noorden and G.J. Patent*

Department of Histochemistry, Royal Postgraduate Medical School, London, England and *Department of Zoology, University of Montana, USA

Summary. Immunocytochemical staining has revealed that the pancreatic islets of various teleost fishes contain a pancreatic polypeptide (PP)-like substance which cross-reacts with antibodies to mammalian and avian PP. The PP cells were frequently found at the periphery of the islets, as in mammals, and were of irregular shape.

Key words: Pancreatic islets – Teleost fishes – Pancreatic polypeptide – Endocrine cells – Immunostaining.

Introduction

It is well established that insulin is produced by B cells and glucagon by A cells in pancreatic islets from fish to mammals. The cells are distinguished by histological stains, ultrastructural morphology of their granules and immunocytochemical staining. D cells are now known to contain somatostatin in all species in which they have been investigated, including some teleost fishes (Johnson et al., 1976).

An additional pancreatic hormone, pancreatic polypeptide (PP), was discovered first in chickens (Kimmel et al., 1968, 1971) and subsequently in mammals (Lin and Chance, 1972). It has been localized to a fourth endocrine cell type scattered in the exocrine parenchyma in birds (Larsson et al., 1974) and both scattered and at the periphery of the islets in mammals (Larsson et al., 1975, 1976; Polak et al., 1976).

At least four cell types, of varying morphology and localization, have been described in the islets of several species of teleost fish (Kobayashi and Takahashi,

Send offprint requests to: Miss S. Van Noorden, MA, Department of Histochemistry, Royal Postgraduate Medical School, Du Cane Road, London W12 OHS, England

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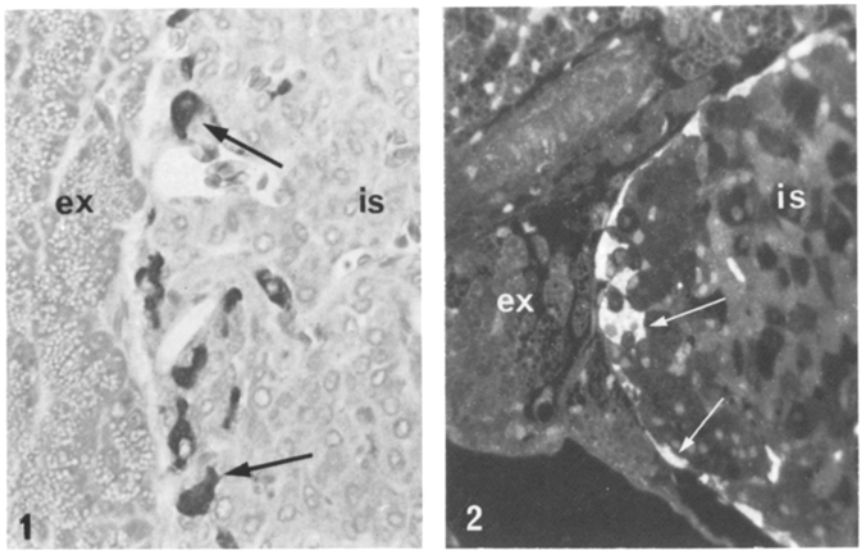

Fig. 1. Pike pancreas. Immunoperoxidase (PAP) reaction with antibody to APP (preabsorbed with glucagon). Bouin-fixed, paraffin-embedded material; 5 micron section. ×756

Fig. 2. Swordtail islet. Immunofluorescence reaction with antibody to BPP (preabsorbed with glucagon and insulin). Glutaraldehyde-fixed, resin-embedded material; 1 micron section. ×388. Arrows show immunoreactive cells. *is* islet; *ex* exocrine pancreas

1973; Thomas, 1970, 1973; Brinn, 1975; Klein and Lange, 1977). Since cross-reactions between antigens and antibodies from different species often occur, it seemed worth while to investigate the possible presence of a PP-like substance in fishes using, initially, immunostaining techniques with antibodies to avian PP (APP) and bovine PP (BPP).

Materials and Methods

Bouin-fixed pancreas or principal islets were obtained from the following species: *Esox lucius* (pike) (3), *Xiphophorus helleri* (swordtail) (5), *Anguilla anguilla* (eel) (2), *Ictalurus nebulosa* (catfish) (1), *Lepisosteus osseus* (garpike) (1) and *Gillichthys mirabilis* (12). In some cases freeze-dried, benzoquinone vapour-fixed, and formalin-fixed or glutaraldehyde-fixed material was also available. Chicken and rat or dog pancreas provided positive control tissue.

The indirect immunofluorescence technique (Coons et al., 1955) and the unlabelled antibody enzyme (peroxidase-anti-peroxidase, PAP) technique (Sternberger, 1974) were used. Anti-APP or BPP was used as the first layer. Controls included absorption of the antibodies prior to staining with glucagon, insulin and PP, singly or in combination, as well as parallel staining with antibodies to glucagon, insulin or somatostatin or with non-immune serum.

Results

Antibodies to both APP and BPP consistently and specifically stained cells which were different from A, B or D cells in the islets of all the species examined except *Gillichthys*.

The PP-like activity was mainly found in angular cells at the periphery of the islets at the junction with the exocrine pancreas (Figs. 1, 2). Occasional reactive cells were seen in the pancreatic ducts in the pike and very rarely in the exocrine parenchyma. In both pike and eel, which have compact pancreases, many sections showed islets which did not contain any PP cells. In *Gillichthys* the reaction was either absent or very weak by comparison with the other fishes in which it was very strong.

The antibody to APP contained some immunostaining cross-reactivity to insulin and glucagon which could be removed by prior absorption of the antiserum with a combination of insulin and glucagon. The remaining activity stained the same cells as the antibody to BPP and could be completely removed by additional absorption with APP. The activity of the antibody to BPP could be abolished by absorption with BPP but not by glucagon or insulin.

Glucagon, insulin and somatostatin-like immunoreactivity were found in the islets of all species examined, in presumed A, B and D cells respectively. Glucagon cells were frequently found in association with the PP cells at the periphery of the islets but occupying a much larger area of the islet, extending towards the centre.

Discussion

Immunostaining with antibodies to mammalian and avian PP has shown that a PP-like substance can be found in some teleost fish pancreatic islets.

The demonstrated cells may well correspond to a fourth cell type such as has been described in the islets of several fishes, eg. cod (Thomas, 1970) and dab (Thomas, 1975) where the fourth cell-type is at the periphery of the islet. Klein and Lange (1977) described a clear cell widely distributed in the islets of *Xiphophorus,* which is evidently not the PP cell since PP staining is confined to the periphery of the islet in this species and thus more resembles their A_2 cell. Similarly the flame-shaped cells of *Lepisosteus* (Epple and Brinn, 1975) cannot contain PP.

There is at present no explanation for the inconsistent staining achieved in *Gillichthys*. Either the principal islet of *Gillichthys* lacks PP or contains a very small amount, or *Gillichthys* PP differs from that of the other fishes examined in being unable to cross-react with antibodies to APP and BPP, or the conditions of fixation for this material were unsuitable for immunostaining PP (although adequate for the other islet hormones).

The staining of glucagon and insulin by the antiserum to APP is explained by the fact the antibody was raised to an extract of chicken pancreas which contained traces of insulin and glucagon as well as PP. These substances would elicit antibody formation which would probably be unimportant at the dilution used in radioimmunoassay, and even in immunostaining the chicken pancreas, but at the higher concentration which had to be used for immunostaining in the heterologous species it was enough to interfere with the stain. The unwanted immunoreactivity could however be removed by prior absorption of the antibody with a combination of glucagon and insulin.

The gastroenteropancreatic hormones of mammals are the subject of intense

investigation, but the physiological role of PP is not yet well established. The gastroenteropancreatic hormones of fishes are as yet little studied.

In addition to the islet hormones, insulin, glucagon and somatostatin, secretin- and cholecystokinin-like activities have been found in intestinal extracts from the eel (Barringtom and Dockray, 1972), pike (Dockray, 1974), and *Chimaera* (Nilsson, 1970). We report here the first demonstration of a PP-like substance in fishes. There is obviously a need for further work on the extraction, purification and analysis of this substance as well as further characterization of its cell of origin by histological and electron microscopical techniques, and its identification in other species of fish, including elasmobranchs, and possibly in other parts of the gut as well as the islets. The finding that antibodies to mammalian or avian PP can be used to localize presumably similar substances in fishes offers great possibilities for the elucidation of some of these problems. The presence of immunologically similar PP-like substances in two classes of vertebrates as far removed from each other as fishes and mammals indicates that a molecule of this type occurred very early in the evolutionary history of the vertebrates, before the separation of the lines leading to fish and mammals. The survival of these presumably related molecules suggests that the PP-like substances are probably of physiological importance.

References

Barrington, E.J.W., Dockray, G.J.: Cholecystokinin-pancreozymin-like activity in the eel (*Anguilla anguilla* L.). Gen. comp. Endocr. **19,** 80–87 (1972)

Brinn, J.E., Jr.: Pancreatic islet cytology of Ictaluridae (Teleostei). Cell Tiss. Res. **162,** 357–365 (1975)

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)

Dockray, G.J.: Extraction of a secretin-like factor from the intestines of pike (*Esox lucius*). Gen. comp. Endocr. **23,** 340–347 (1974)

Epple, A., Brinn, J.E. jr.: Islet histophysiology: evolutionary correlations. Gen. comp. Endocr. **27,** 320–349 (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)

Kimmel, J.R., Pollock, H.G., Hazelwood, R.L.: Isolation and characterization of chicken insulin. Endocrinology **83,** 1323–1330 (1968)

Kimmel, J.R., Pollock, H.G., Hazelwood, R.L.: A new polypeptide hormone. Fed. Proc. **30,** 1318 (abstract) (1971)

Klein, C., Lange, R.H.: Principal cell types in the pancreatic islet of a teleost fish, *Xiphophorus helleri* H. A correlative light and electron microscopical investigation. Cell Tiss. Res. **176,** 529–552 (1977)

Kobayashi, K., Takahashi, Y.: Fine structure of Langerhans' islet cells in a marine teleost *Conger japonicus* Bleeker. Gen. comp. Endocr. **23,** 1–18 (1974)

Larsson, L.-I., Sundler, F., Håkanson, R.: Immunohistochemical localization of human pancreatic polypeptide to a population of islet cells. Cell Tiss. Res. **156,** 167–171 (1975)

Larsson, L.-I., Sundler, F., Håkanson, R.: Pancreatic polypeptide – a postulated new hormone: identification of its cellular storage site by light and electron microscopic immunocytochemistry. Diabetologia **12,** 211–226 (1976)

Larsson, L.-I., Sundler, F., Håkanson, R., Pollock, H.G., Kimmel, J.R.: Localization of APP, a postulated new hormone, to a pancreatic endocrine cell type. Histochemistry **42,** 377–382 (1974)

Lin, T.N., Chance, R.E.: Spectrum gastrointestinal actions of a new bovine pancreas polypeptide (BPP). Gastroenterology **62,** 852 (abstract) (1972)

Nilsson, A.: Gastrointestinal hormones in the holocephalian fish (*Chimaera monstrosa* L.) Comp. Biochem. Physiol. **32,** 387–390 (1970)

Polak, J.M., Adrian, T.E., Bryant, M.G., Bloom, S.R., Heitz, Ph., Pearse, A.G.E.: Pancreatic polypeptide in insulinomas, gastrinomas, vipomas, and glucagonomas. Lancet **1976 I,** 328–330

Sternberger, L.: Immunocytochemistry. Englewood Cliffs, N.J.: Prentice Hall 1974

Thomas, N.W.: Morphology of the endocrine cells in the islet tissue of the cod *Gadus callarias*. Acta endocr. (Kbh.) **63,** 679–695 (1970)

Thomas, N.W.: Observations on the cell types present in the principal islet of the dab, *Limanda limanda*. Gen. comp. Endocr. **26,** 496–503 (1975)

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