Identification of the D₁-cell as the source of human pancreatic polypeptide (HPP)

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SUMMARY Immunoperoxidase positive HPP-producing cells have been examined ultrastructurally using the serial semithin-thin section technique. The features of the HPP cells were found to be identical with those of the previously described D₁-cell. The D₁-cells are scattered throughout the exocrine parenchyma and occur to a minor extent in the islets.

HPP is a straight chain 36-residue peptide. Homologous peptides have been isolated from avian, bovine, porcine, and ovine pancreas. PP can be detected by radioimmunoassay in the plasma of different species, including man (Kimmel *et al.*, 1968, 1975; Lin and Chance, 1972, 1974; Langslow *et al.*, 1973; Floyd *et al.*, 1975).

PP-containing cells in bird and mammal have been observed to occur mostly in the pancreas (Larsson *et al.*, 1974, 1975b). In the human, PP cells have merely been identified 'as cells sharing many features with known peptide hormone-producing cells' and being 'distinct from the A, B and D cells' at the ultrastructural level (Larsson *et al.*, 1975a). The D₁-cell has been recognised for several years but no convincing identification of its possible hormonal product has been published. In this paper we present evidence that the D₁-cell is the PP-producing cell in man.

Methods

Small blocks of human pancreas obtained at surgery for gastric cancer were fixed in a mixture of paraformaldehyde (2%), saturated picric acid (0.2%), and sucrose (0.1 M), in phosphate buffered saline (PBS) pH 7.2 at +4°C for 48 hours. After thorough washing the blocks were dehydrated in alcohol and acetone and embedded in Epon 812. Serial semithin-thin sections were cut (Polak *et al.*, 1975). After removal of the Epon by absolute ethanol saturated with NaOH (Lake and Europa, 1965), HPP cells were localised by an indirect immunoperoxidase method in the semithin section. Rabbit anti-HPP

serum and a peroxidase-labelled porcine anti-rabbit-IgG-globulin serum were used as first and second layers respectively. The peroxidase was subsequently localised using as substrate a solution of 3,3'-Diaminobenzidine-tetrahydrochloride (Sigma; 0.5 mg/ml) and H₂O₂ (0.01%) in an 0.05 M Tris-HCl-buffer (pH 5.0). After fixation with OsO₄ (1%) in PBS (pH 7.2) the sections were dehydrated and mounted. Cells displaying a positive reaction were subsequently localised in the adjacent thin sections. Some tissue blocks were conventionally fixed in glutaraldehyde (3%) in PBS (pH 7.2), and OsO₄ (1%) in PBS (pH 7.2) and embedded in Epon. All thin sections were stained with uranyl acetate and lead citrate.

The anti-HPP serum used in this study was raised as described previously (Polak *et al.*, 1976) and did not show any cross-reaction with insulin, glucagon, gastrin, or vasoactive intestinal peptide in radioimmunoassay. PP-immunoreactivity in extracts of normal human pancreas eluted from a Sephadex G50 column in a position identical with pure HPP suggesting that it is similar to or identical with the normally produced hormone (Adrian *et al.*, unpublished observations). Control reactions, which were invariably negative, included incubation with normal rabbit serum and with rabbit anti-HPP serum absorbed with excess pure HPP (100 µg/ml diluted antiserum). Absorption of the anti-HPP serum with somatostatin, gastrin, insulin, or glucagon (100 µg/ml diluted antiserum) before incubation did not alter the pattern of distribution of positive cells, whereas incubation of the sections with antisera to insulin, glucagon, and somatostatin resulted in the pattern characteristic for the distribution of B-, A-, and D-cells respectively.

Fig. 1 (a) Group of HPP cells in the exocrine pancreas. Semithin section, indirect immunoperoxidase stain. $\times 1560$. (b) Adjacent section. Cell (marked with arrow in Fig. 1a) with small secretory granules. Note adjacent exocrine cells. $\times 8125$. (c) Same section as Fig. 1b. Small secretory granules with a dense core and a tightly fitting membrane in the cell marked with a ring in Fig. 1a. $\times 23\,562$. (a)-(c), Paraformaldehyde-picric acid-sucrose fixation.

Results

In a series of 25 samples of human pancreas HPP cells were regularly found to be scattered throughout the exocrine parenchyma, relatively few cells being located in the islets. The cells located in semithin sections (Fig. 1a) were found by electron microscopy to contain a large number of small secretory granules with a very electron-dense core (Fig. 1b and c). After conventional EM fixation the cells are characterised by round, or occasionally oval, secretory granules with a uniformly electron-dense core generally

closely applied to the membrane. At times a very narrow electronlucent space may be observed between the core and the membrane (Fig. 2). The mean diameter of the granules is 170 nm (range 100-260 nm; diameter of 500 granules determined).

Discussion

The features of the HPP-producing cell described above are those of the D₁-cell (revised Wiesbaden nomenclature: Solcia *et al.*, 1973). This corresponds, presumably, to cell type IV of Munger (1972) and to

Fig. 2 Small round secretory granules with a dense core. The limiting membrane is tightly fitting except in some granules in which a narrow space between the core and the membrane can be seen. Glutaraldehyde-osmium-tetroxide fixation, $\times 15\,325$.

cell type V of Deconinck *et al.* (1971). On the basis of its localisation the D₁-cell can easily be distinguished from the other endocrine cells of the pancreas. A-, B-, and D-cells—producing glucagon, insulin, and somatostatin respectively—occur in the islets in a characteristic distribution pattern (Orci and Unger, 1975). At the ultrastructural level, however, the secretory granules of the D₁-cell resemble, at first glance, those of the A-cell. Closer inspection reveals that the mean diameter of the A-cell granules is 300 nm (range 250–450 nm; diameter of 500 granules determined), indicating a totally separate cell population. Furthermore, the A-cell granules almost invariably exhibit an eccentric, round, very electron-dense core surrounded by a semilunar, less dense, granular area all enclosed by a tightly fitting membrane. Evidence is accumulating that HPP can be considered as the fourth pancreatic hormone in addition to insulin, glucagon, and somatostatin. It can be extracted from the normal human pancreas, is released into the bloodstream after food intake in man (Floyd *et al.*, 1975; Adrian *et al.*, unpublished observations), and its specific cellular origin has now been defined.

The location of the D₁-cells throughout the exocrine parenchyma in the human pancreas immediately suggests a role for HPP in the regulation of exocrine pancreatic secretion. Administration of bovine pancreatic polypeptide (BPP) has been found to influence pancreatic bicarbonate and enzyme secretion in the dog and at higher doses to affect gastric acid output and sphincter tonus (Lin and Chance, 1974) but no studies have yet been under-

taken in man. As HPP is released into the blood after food intake it may be anticipated that it is involved in normal gut physiology.

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