Electron immunohistochemical evidence for the human intestinal I cell as the source of CCK

ALISON M. J. BUCHAN, JULIA M. POLAK, E. SOLCIA, C. CAPELLA, D. HUDSON, AND A. G. E. PEARSE

From the Department of Histochemistry and Endocrine Unit, Royal Postgraduate Medical School, Hammersmith Hospital, London, and Centre of Histopathology, Histochemistry and Ultrastructure, University of Pavia at Varese, Italy

SUMMARY Evidence was obtained by the use of alternate semithin-thin serial sections for light and electron microscopy that the I cell is the source of CCK-PZ. The antibodies used were raised to a synthetic fragment of the mid part (9-20) of the (1-33) CCK-PZ molecule, and were thus free from any contamination with cross-reacting subpopulations of antibodies that might bind to gastrin.

Cholecystokinin-pancreozymin (CCK-PZ), a biologically active peptide, has been shown by immunohistochemistry to be present in the duodenal and jejunal areas of the human small intestine (Buffa et al., 1976; Polak et al., 1975). Clearly it is important to identify the endocrine cell responsible for the synthesis and storage of this hormone. It has been previously suggested that the ultrastructurally identified I cell is the source of CCK-PZ (Solcia et al., 1975). Early evidence for this association was circumstantial, in that the distribution of CCK-PZ activity and the distribution of endocrine cells immunostained with antibodies raised to the whole molecule of CCK-PZ corresponded to the distribution of the morphologically identified I cells (Polak et al., 1975; Buffa et al., 1976). Although the I cell origin of CCK-PZ was confirmed to some extent by preliminary results using the semithin-sectioning method (Polak et al., 1975), it was necessary to produce more conclusive evidence for the final identification of the cellular origin. There is, unfortunately, cross-reactivity between gastrin and antisera raised to the whole molecule of CCK-PZ because the two peptides share a C-terminal sequence (Jorpes and Mutt, 1973). Thus antibodies raised to the whole CCK-PZ molecule will contain a population of antibodies capable of binding this C-terminal sequence and therefore cross-reacting with any gastrin present in the tissue. This has been a major problem in the immunohistochemical identification of CCK-PZ, as cells containing gastrin are also found in the duodenal mucosa (Polak et al., 1975; Buffa et al., 1976).

Immunohistochemical techniques use much higher concentrations of antibodies than radioimmunoassay techniques, so it is possible for even a subpopulation of antibodies to create a positive staining result. Initially, the only means of abolishing this staining effect was to absorb the CCK-PZ antibodies with pentagastrin to remove the cross-reacting antibodies while leaving the specific antibodies unaffected (Buffa et al., 1976).

In this study we have achieved the complete identification of the CCK-PZ cell in the human small intestine by use of antibodies to a synthetic fragment of CCK-PZ (9-20) which lacks the sequence homology with gastrin.

Methods

Twenty-five surgical samples of human antrum, duodenum, and jejunum were fixed by two techniques: (1) 2.5% purified glutaraldehyde in 0.05 M phosphate buffer pH 7.3 for five minutes (used for immunohistochemical techniques), and (2) 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 for two hours, postfixed in osmium tetroxide (for conventional electron microscopy). The samples were then dehydrated through graded alcohols and embedded in Araldite. Sections were taken serially, first 1µm then 60 nm. The 1µm sections were used for the immunohistochemical technique using an indirect immunofluorescent method as outlined
jejunal mucosa below. The adjacent 60 nm sections were floated onto copper grids for counterstaining with uranyl acetate and lead citrate.

The Araldite was removed from the 1µm sections by saturated NaOH in ethanol. The sections were rehydrated through graded alcohols, then the indirect immunofluorescent technique of Coons, Leduc and Connolly (Coons et al., 1955) was applied. The first layer was CCK-PZ (9-20) antiserum at a dilution of 1:400 for a 24 hour incubation. The second layer was FITC goat antirabbit conjugate (Hyland) for one hour at room temperature. Various controls were applied, including prior absorption of the antibodies with the following antigens: CCK-PZ (1-33) (99% pure from Professor V. Mutt 3000Ivy dog units per mg), CCK-PZ (9-20) synthetic (Polak et al., 1977), CCK-PZ (26-33) Dr M. Ondetti, Squibb Institute for Medical Research, New Jersey, USA) Gastrin 1-17 synthetic (ICl), somatostatin (synthetic, cyclic from Beckman Bioproducts), GIP-porcine GIP 99-9% pure from Professor J. C. Brown, Vancouver, and glucagon (10units/10 mg, Eli Lilly, Indianapolis, USA). The dodecapeptide Met-Ile-Lys-Asn-Leu-Gln-Ser-Leu-Asp-Pro-Ser-His, corresponding to the midportion (9-20) of the CCK-PZ molecule, has been synthesised (Polak et al., 1977) and used to raise specific antibodies.

Rabbits were injected with 100 µg dodecapeptide coupled to ovalbumin by the glutaraldehyde method in complete Freund’s adjuvant. Animals were injected at three monthly intervals (Polak et al., 1977) for nine to 12 months. The specificity of the antibodies was tested by absorption with the peptides before staining as already stated. It was also checked by the recently developed Enzyme Linked Immunosorbent Assay (ELISA) (Voller et al., 1976), a technique applied here for the first time to gut hormones, although it has been extensively used in other fields.

We measured the diameter of all granules found in the CCK-PZ cells identified ultrastructurally by immunocytochemistry and in I cells identified by conventional electron microscopy. The mean diameter was calculated and corrected for sectioning artefacts with the formula \( D = \frac{4}{\pi} \cdot d \) (Baetens et al., 1976).

Results

Scattered endocrine cells within the duodenal and jejunal mucosa were specifically stained by the CCK-PZ (9-20) antibodies in semithin sections (Fig. 1a).

Quenching of the antibody to the mid portion fragment of CCK-PZ occurred only with CCK-PZ (1-33) and (9-20) molecules and not with gastrin (1-17), glucagon, somatostatin, GIP, and 26-33 CCK-PZ octapeptide. The ELISA technique showed that the antibodies raised to the synthetic fragment of CCK-PZ tested at the same dilution as used in the immunocytochemical staining, bound only to the 1-33 and 9-20 CCK-PZ sequences and not to the other peptides. A sample of an antiserum raised to the whole CCK-PZ (1-33) molecule (Polak et al., 1975) showed binding to both the CCK-PZ peptides and to gastrin 1-17. In addition, the anti-CCK-PZ (9-20) serum, when applied to sections of human antrum failed to show any positive staining, whereas serial sections stained with gastrin 1-17 antiserum showed numerous positive cells. The CCK-PZ cells which were identified in the semithin sections were identified at the ultrastructural level on consecutive sections (Fig. 1b and c). They constituted a homogeneous population of cells with mainly round, sometimes angular, relatively dense granules averaging 260 nm (SD ± 22 nm) in size, corrected values: 330 nm (700 granules from nine cells measured). I cells as identified by conventional electron microscopy (Fig. 2) also showed mainly round, compact granules with closely applied membrane, measuring 253 nm (SD ± 58 nm) corrected values 322 nm (604 granules measured from six cells). The two measurements did not differ significantly. Although some G cells with vesicular granules of floccular content were found by conventional electron microscopy in the human duodenum, none of the cells reacting with the specific CCK-PZ antibodies resembles G cells.

Discussion

These results show that, when structurally similar hormones with overlapping properties are found in the same area of the gut, the complexity of the situation can be resolved by combined light and electron immunohistochemical techniques using antibodies to fragments of one hormone molecule which lack a common sequence with other peptides.

Immunocytochemical findings using the specific CCK-PZ (9-20) antibodies permitted the staining of the CCK-PZ cell without interference from gastrin cells.

Although C-terminal peptides of CCK-PZ (which are reported to be quite abundant in the small intestine (Larsson et al., 1978; Rehfeld, 1978) were not recognised by the antibodies in control tests, antibodies to the whole 1-33 CCK-PZ molecule proved heavily reactive and ensured detection of CCK-PZ cells, although any gastrin cells present would of course be stained in addition. The ultrastructural features of most of the cells stained by
Electron immunohistochemical evidence for the human intestinal I cell as the source of CCK

Fig. 1 (a) CCK-PZ cell of the human jejunal mucosa stained with specific antibodies to CCK-PZ fragment 9-20, ×625. (b) Electron micrograph of the same cell, ×4000. (c) Detail of the same to show characteristic secretory granules, ×20,000.

this antibody corresponded closely to those already reported for human I cells. Although it is possible that cells storing only the C-terminal fragments with no 9-20 CCK-PZ content might have escaped detection in our immunohistochemical staining using the CCK-PZ 9-20 antibodies, it must be stressed that the existence of such cells has never been demonstrated. The I cell was first identified in the dog (Bussolati et al., 1971) as a cell with granules of size and structural pattern intermediate between those of S and L cells, which are reputed to produce respectively secretin and glucagon-like immunoreactivity (GLI). In man, the identification of I cells on ultrastructural grounds alone is made more difficult by the smaller size of human L cell granules; however, granules of I cells are slightly more electron dense than those of L cells and lack their thin argyrophil halo (Capella et al., 1972). Moreover, human I cells—like CCK-PZ cells (Polak et al., 1975; Buffa et al., 1976) and unlike L (GLI) cells (Grimelius et al., 1976)—are well represented in the duodenum but lacking in the colon and rectum (Capella et al., 1976). The results of the present immunohistochemical investigation allow us to
confirm the ultrastructural localisation of the human I cell on a sounder basis and to identify it with the CCK-PZ cell of light microscopy. It should now be possible to carry out ultrastructural studies of this cell in experimental and pathological conditions.

We are very grateful to Dr A. Voller and his colleagues at the Nuffield Institute of Comparative Medicine for their help in setting up the ELISA system for use in our study. The gift of 99% pure CCK-PZ from Professor V. Mutt is also gratefully acknowledged. This work was made possible with the aid of grants from the Medical Research Council, the Nuffield Research Campaign, the Volkswagenwerk Stiftung (Hannover), and Consiglio Nationale delle Ricerche (Rome).

References


Electron immunohistochemical evidence for the human intestinal I cell as the source of CCK


