

Cellular localization of gastric inhibitory polypeptide in the duodenum and jejunum

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SUMMARY Indirect immunofluorescence studies using an antiserum to purified porcine gastric inhibitory polypeptide indicate, in the gastrointestinal tract of dog and man, that this polypeptide is present in cells situated predominantly in the mid-zone of the glands in the duodenum and, to a lesser extent, in the jejunum. Absolute correlation of the gastric inhibitory polypeptide cell with one or other of the known endocrine-like cells identified by electron microscopy awaits confirmation by electron immunocytochemistry. It is here identified as an endocrine polypeptide cell of the APUD series and, provisionally, as the D₁ cell. While the hormonal status of a given polypeptide depends ultimately on physiological experiments the present results strengthen the view that gastric inhibitory polypeptide is indeed a hormone.

In 1969 Brown, Pederson, Jorpes, and Mutt described an enterogastrone, extractable from porcine intestine, which strongly inhibited gastric acid secretion. The purification of an apparently similar enterogastrone was reported in the same year by Lucien, Itoh, Sun, Meyer, Carlton, and Schally. The first of these polypeptides, named gastric inhibitory polypeptide (GIP) by Brown, Mutt, and Pederson (1970), was later shown, with the publication of the complete sequence by Brown and Dryburgh (1971), to contain 43 amino acid residues. Similarities in its structure to both porcine glucagon and porcine secretin were noted. Fifteen of the first 26 amino acids occur as in the former and nine of the first 26 are in the same position as in secretin. The 17 C-terminal residues are not common to any other intestinal polypeptide.

The cellular localization of only two of the polypeptide hormones of the intestine has so far been recorded. These are secretin, found in the S cell of the duodenum by Bussolati, Capella, Solcia, Vassallo, and Vezzadini (1971) and, independently, by Polak, Bloom, Coulling, and Pearse (1971a), and enteroglucagon which was located in the L cell of the intestine by Polak, Bloom, Coulling, and Pearse (1971b).

We report here the results of immunofluorescence studies on the localization of GIP in human and canine intestine.

Material and Methods

Operative samples of duodenal and jejunal mucosa from seven human subjects were studied. Two of these had gastric carcinoma with very low gastric acid levels.

Canine antral, duodenal, and jejunal mucosa samples were obtained from two mongrel puppies, 8 and 12 weeks old respectively.

In both species small pieces of mucosa were processed in a variety of different ways, as indicated below.

IMMUNOFLUORESCENCE

Three processing schedules were employed.

1 Fixation in cold 4% methanol-free formaldehyde for periods between three hours and five days (Polak, Bussolati, and Pearse, 1971). After washing out excess fixative part of the tissue was quenched at -158° in Arcton (Freon) 22 and subsequently used for the provision of cryostat sections. These were picked up on gelatine-formaldehyde-coated slides. The remaining tissues were dehydrated in graded alcohols and embedded in paraffin wax. Sections were allowed to dry, at 37°, for 24 to 48 hours.

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2 Fixation in 10% carbodiimide (Kendall, Polak, and Pearse, 1971): this fixative is prepared by dissolving, immediately before use, 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide hydrochloride (Sigma) in 0.1M phosphate buffer saline at pH 7.1. Tissues were fixed for three hours and then washed in several changes of 0.1M phosphate buffer (pH 7.1), containing 7.5% sucrose, for at least 24 hours. As after methanol-free formaldehyde, both cryostat and paraffin sections were employed.

3 Selected pieces of mucosa were quenched immediately after removal in Arcton (Freon) 22 at -158° . Some of these were used for the preparation of unfixed cryostat sections, others were freeze-dried for 12 to 18 hours at -40° in a thermoelectric dryer. Subsequently these pieces were embedded *in vacuo* in 56° paraffin wax. Sections ($5\ \mu\text{m}$) were mounted on glycerine-albumin-coated slides and dried at 37° for 24 to 48 hours.

Sections processed by each of these schedules were used for an indirect immunofluorescence procedure (Coons, Leduc, and Connolly, 1955). Control sections were invariably employed, as described by Polak *et al* (1971a and b), in order to check the specificity of the immune reactions. The antibodies used were raised in guinea-pigs against a highly purified GIP and fluorescein-labelled rabbit anti-guinea-pig globulin was obtained commercially (Hyland).

Fluorescence microscopy was carried out using a Zeiss (Oberkochen) standard universal microscope with HBO200 lamp, BG12 and BG38 excitation filters, and a K530 barrier filter (50% transmission at about 530 nm). Photomicrographs were taken on Ilford FP4 film.

DARK-FIELD OBSERVATIONS

Fresh frozen cryostat, or freeze-dried paraffin sections, were studied for dark-field luminescence, which reveals pancreatic A cells (Cavallero and Solcia, 1964) and intestinal enteroglucagon cells (Polak *et al*, 1971b).

CYTOCHEMICAL AND STAINING REACTIONS

Small pieces of tissue from each region were fixed for 24 hours in the following fixatives: 6% glutaraldehyde in 0.1M phosphate buffer pH 7.4; Bouin's fluid; glutaraldehyde-picric acid (Solcia, Vassallo, and Capella, 1968). All samples were washed in water, dehydrated, cleared, and embedded in 56° paraffin wax. Sections ($5\ \mu\text{m}$) from appropriately fixed blocks were processed by methods selective for the secretory granules of gastrointestinal endocrine cells.

Sections were stained with 1% aqueous toluidine blue at pH 5.0, with and without treatment with

mineral acid (0.2N HCl, 60° for 30 minutes to two hours); by the lead haematoxylin method (Solcia, Capella, and Vassallo, 1969); by the xanthidrol method for tryptophan and 5-hydroxytryptamine (Lillie, 1957); the Masson-Fontana method for enterochromaffin granules (Pearse, 1972); and the silver impregnation technique of Grimelius (1968).

ELECTRON MICROSCOPY

Small blocks of tissue were fixed, immediately after removal, in 3% glutaraldehyde in 0.1M phosphate buffer at pH 7.6 for two hours at 4° . Excess fixative was removed by repeated washing in 0.1M phosphate buffer containing 0.1M sucrose. They were then dehydrated in ethanol and ethoxypropane and finally embedded in Araldite mixture. With some blocks, a double fixation procedure was carried out, using postfixation with osmium tetroxide at 4° for two hours (Millonig, 1962). Sections were stained by lead citrate and uranyl acetate and viewed in an AEI EM 6B electron microscope.

Results

Immunofluorescence studies showed that GIP cells were relatively numerous by comparison with the number of S (secretin) cells found in identical areas of the intestine. They were localized predominantly in the middle zone of the glands, in the duodenum, and to a lesser extent in the jejunum, in both dog and man (figs 1 and 2). There were a few GIP cells in the

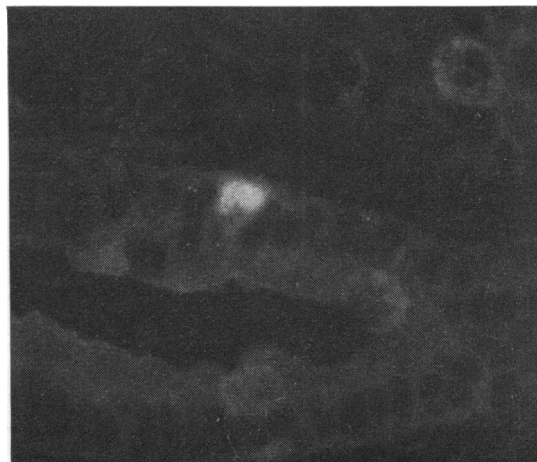


Fig. 1 Indirect immunofluorescence reaction using guinea-pig antiporcine GIP serum and fluorescein-labelled sheep anti-guinea pig IgG. Dog duodenum. In this longitudinal section (MFF-fixed) through the duodenal glands a single GIP cell is visible. Its granules are concentrated on the site of the cell adjacent to the basement membrane. $\times 750$

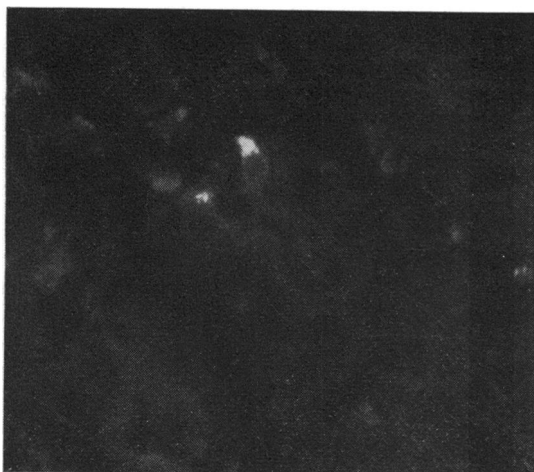


Fig. 2 As figure 1. Human duodenum. Transverse section. Shows a single basigranular GIP cell. $\times 450$

duodenal villi, but not in the jejunal villi. Human GIP cells were most easily found in duodenal samples from the two cases with low gastric acidity, and least easily found in cases of duodenal ulcer.

Cytochemical and staining reactions distinguish a number of endocrine cell types in the gastrointestinal tract. The GIP cell was characterized as lead haematoxylin-positive, Grimelius-positive, non-argentaffin and weakly positive for tryptophan. It

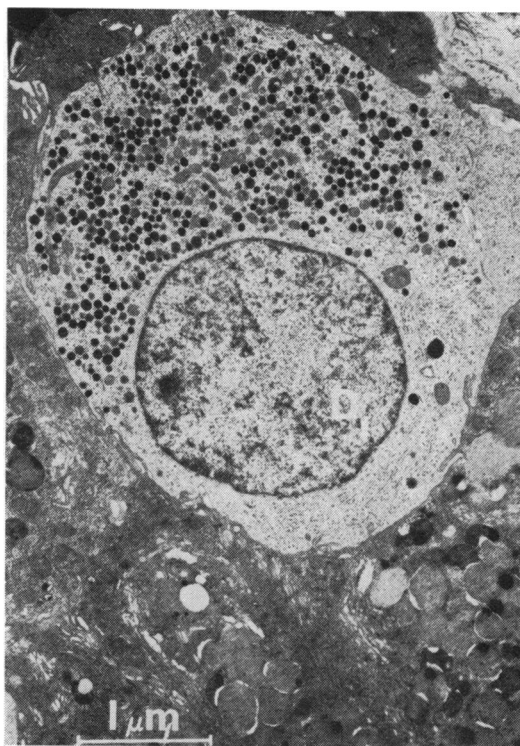


Fig. 4 Electron micrograph, human duodenum: a single D_1 cell containing small granules of variable electron density, without visible halo. $\times 5500$

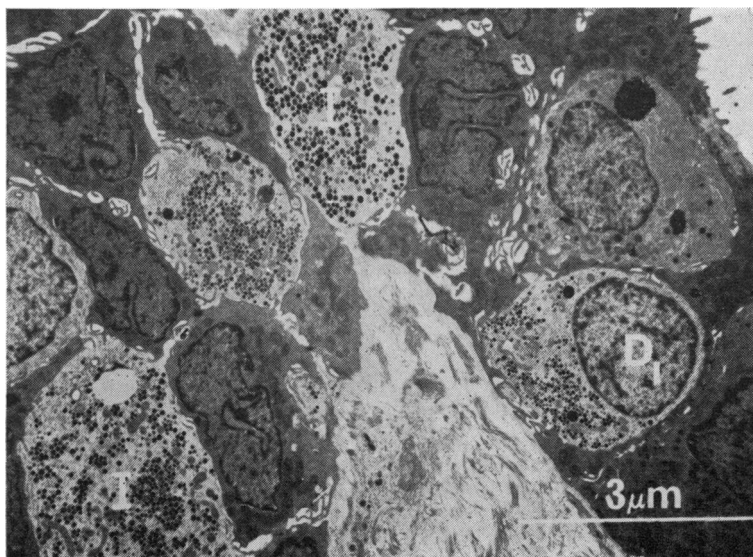


Fig. 3 Electron micrograph, human duodenum: parts of six endocrine cells are present, two are identified as D_1 cells and two as I (intermediate) cells. $\times 4000$

was considered to belong to the APUD series of endocrine polypeptide cells (Pearse, 1969) although its remaining APUD characteristics have not yet been determined.

In the glands of duodenum and jejunum electron microscopy likewise revealed a number of different types of endocrine cell (fig 3). In the first part of the duodenum, the predominant site for immunofluorescent GIP cells, the cell whose distribution most closely matched the latter was the small granule-containing D₁ cell (fig 4) described by Vassallo, Capella, and Solcia (1971) and Vassallo, Solcia, Bussolati, Polak, and Pearse (1972). We observed also very numerous enterochromaffin (EC) cells, D cells, S cells, I cells and, in the duodenum, an occasional G (gastrin) cell.

Discussion

Enterogastrones can be defined as hormones, released from the intestinal mucosa by the introduction into the lumen of acid or hypertonic solutions. Pederson and Brown (1972) recently showed that GIP possesses potent enterogastrone activity but they recommended, with propriety, that until shown to be released into the blood stream by physiological mechanisms it should not be called a hormone.

Identification of the GIP cell as a distinct member of the growing band of gastrointestinal endocrine polypeptide (APUD) cells follows directly on the identification of GIP or a GIP-like polypeptide in tumour material from a case of the watery diarrhoea, hypokalaemia, achlorhydria syndrome (Elias, Polak, Bloom, Pearse, Welbourn, Booth, Kuzio, and Brown, 1972). The cell which we tentatively identify by electron microscopy as the GIP cell (the D₁ cell) is clearly to be distinguished from the D-like (that is to say pancreatic D cell-like) cell described in the canine pylorus by Fujita and Kobayashi (1971). This cell was found to discharge its granules in response to an acid stimulus and hence it may be presumed to produce an enterogastrone. The greater degree of granulation of the GIP cell in our cases with low gastric acidity, and the lower degree of granulation found in duodenal ulcer cases, similarly may be physiological expressions of the role of the cell as the producer of one of the enterogastrones.

Absolute identification of the source of a polypeptide hormone at the ultrastructural level depends on successful application of the techniques of electron microscopical immunology. For the GIP cell this has not yet been achieved. Our findings nevertheless indicate that GIP is produced by an endocrine polypeptide cell, situated in the duodenum and jejunum and distinct from both the S cell

(secretin) and the L cell (enteroglucagon). They therefore support the proposition that GIP is a true hormone.

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Addendum

Subsequent to the submission of this paper we have prepared antisera to pure porcine vasoactive intestinal polypeptide (VIP). The GIP cells showed no cross-reactivity with three different anti-VIP sera.