Histochemistry 67, 221–224 (1980)

Histochemistry

Short Communication

A Pancreatic Polypeptide (PP)-Like Immunoreactant is Present in the Glicentin-Containing Cell of the Cat Intestine*

M. Ravazzola and L. Orci

Institute of Histology and Embryology, University of Geneva Medical School, CH-1211 Geneva 4, Switzerland

Summary. BPP-like immunoreactivity was identified in the intestinal mucosa of the cat. In light microscopy BPP immunoreactive cells were found identical to glicentin-containing cells or L-cells. By immunoelectronmicroscopy, BPP-like material was localized within the glicentin-containing secretory granules.

Introduction

We have recently demonstrated that the source of the gut-GLI-1, glicentin, purified from the porcine digestive tract (Sundby et al. 1976), is the L-cell of the small and large intestine (Ravazzola et al. 1979a, b). In the present work, we provide evidence for the presence in this cell type of a pancreatic polypeptide-like immunoreactant which is stored together with glicentin in the L-cell secretory granules.

Materials and Methods

Samples of cat pancreas and gastro-intestinal tract were fixed for 12 h in Bouin's fluid, washed overnight in tap water, dehydrated in alcohol and embedded in paraffin. Small blocks of the same tissues were fixed in phosphate buffered 4% glutaraldehyde, dehydrated and embedded in Epon 812.

Light Microscopy. Sections of paraffin or Epon embedded material were treated by the indirect immunofluorescence technique of Coons et al. (1955) after removal of the embedding medium (Maxwell 1978). Sets of two consecutive sections were incubated for 2 h at room temperature in a moist chamber with rabbit anti-glicentin serum R64 (1:100) or with rabbit anti-bovine pancreatic polypeptide (BPP) serum nr. 615 R 110 146-6 (1:200), followed by washing in PBS and exposure for 1 h to sheep anti-rabbit IgG coupled to FITC (1:100). The anti-glicentin serum was a gift

* This work was supported by grant nr. 3.120.77 from the Swiss National Science Foundation

0301-5564/80/0067/0221/$01.00

of Dr. A.J. Moody, Novo, Bagsvaerd, Denmark and the anti-BPP serum was a gift of Dr. R. Chance (Lilly Research Laboratory, Indianapolis, USA). Sections were stained with 0.01% Evans blue and examined with a Leitz fluorescence microscope. Controls of specificity were performed by omitting the primary antiserum or by applying the antisera preabsorbed with the homologous or the heterologous antigen (2 mg/ml of undiluted antiserum).

Immunoelectronmicroscopy. Thin sections collected on nickel grids were treated by the technique of Roth et al. (1978). Briefly, sections incubated 2 h at room temperature with anti-glicentin or with anti-BPP serum were rinsed in PBS and floated 1 h on drops of protein A-gold (pAg) complex (1:5). After washing in PBS and distilled water, sections were counterstained with uranyl acetate and lead citrate and examined with a Philips EM 301.

Results

When consecutive semithin sections of cat ileum and colon were stained to reveal glicentin and pancreatic polypeptide the same population of endocrine cells were fluorescent in both cases (Fig. 1 a, b). Previous adsorption of anti-glicentin with glicentin or of anti-BPP with BPP abolished the specific immunofluorescence. In contrast, immunoreactive cells were still present on sections treated with the anti-glicentin serum mixed with BPP or with anti-BPP serum pre-incubated with glicentin.

By using the pAg technique at the ultrastructural level it was found that the antigenic sites reacting with anti-glicentin or anti-BPP antisera were both located in the secretory granules of the L-cell (Fig. 1 c, d). At variance with the results obtained when glutaraldehyde-fixed material was used, sections of Bouin-fixed, paraffin embedded ileum and colon did not contain any BPP immunoreactive cells, although large numbers of glicentin-positive cells were present. In the pancreas, distinct glicentin and BPP immunoreactive cells were found, independently of the fixation used.

Discussion

The present study demonstrates that a BPP-like immunoreactant is present together with glicentin in the intestinal L-cell of the cat. This material probably differs from authentic pancreatic polypeptide since it is detectable only under certain fixation conditions, such as the fixation with glutaraldehyde used in this study or with picric-acid-formaldehyde mixture (Stefanini et al. 1967) as reported by Buffa et al. (1978) for the human large intestine. The fact that pre-incubation of anti-BPP serum with glicentin did not prevent immunostaining indicates that the sequence recognized by the anti-BPP serum may not be present in glicentin molecule, at least as it has been isolated and purified. Conversely, the ability of BPP to abolish the anti-BPP serum immunoreaction favors the idea that, with some types of fixation, a pancreatic polypeptide-like immunoreactant is unmasked or preserved in the intestinal L-cell. Since this material is clearly confined to the secretory granules, it is presumably released by the L-cell together with glicentin.

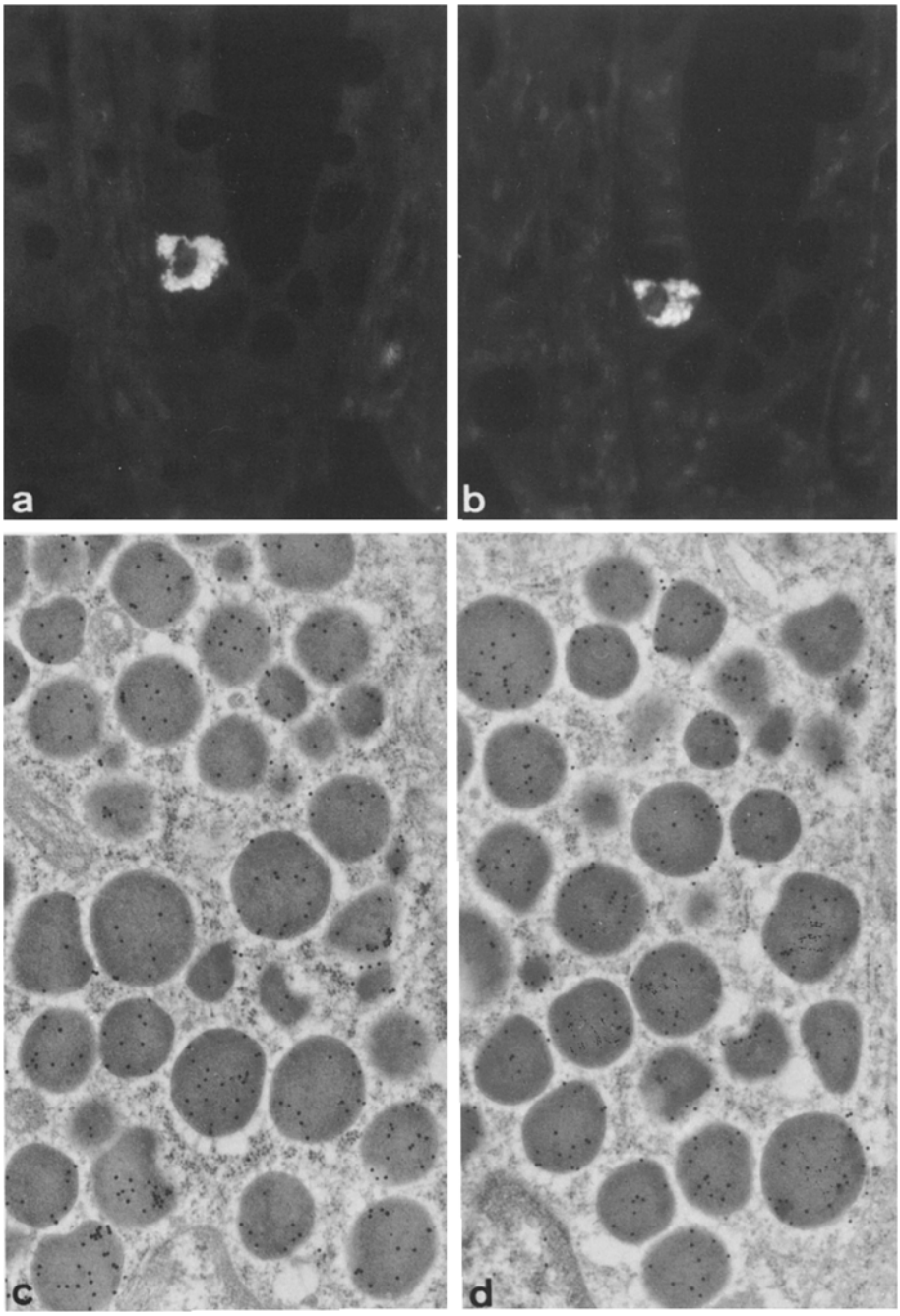

Fig. 1 a, b. Consecutive semithin sections of cat colon treated by immunofluorescence with **a** anti-BPP and **b** anti-glicentin serum. The same cell is immunoreactive to each antiserum. Glutaraldehyde fixation. × 650

Fig. 1 c, d. Thin sections of cat colon after incubation with **c** anti-BPP or **d** anti-glicentin serum and protein A-gold (*pAg*) complex. In both instances, gold particles indicating antigen-antibody reaction sites are located over the L-cell secretory granules. Glutaraldehyde fixation. × 21,000

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Received March 3, 1980

Note added in proofs.

When this paper was in press, similar results have been obtained in human intestine by: Fiocca R, Capella C, Buffa R, Fontana P, Solcia E, Hage E, Chance RE, Moody AJ (1980) Glucagon-, glicentin- and pancreatic polypeptide-like immunoreactivities in rectal carcinoids and related colorectal cells. Am J Pathol (in press).