Rapid Publications

Transformation of Glicentin-containing L-Cells into Glucagon-containing Cells by Enzymatic Digestion

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SUMMARY

Exposure of sections of ileal mucosa to enzymatic digestion with trypsin and carboxypeptidase B reveals a population of immunofluorescent cells after incubation with a specific C-terminally directed antiglucagon serum. These cells, unreactive before enzyme treatment, were identified as L-cells by their immunoreactivity to antiglicentin serum and to cross-reacting (N-terminal) antiglucagon sera. The presence in the L-cells of antigenic sites characteristic of the glucagon-containing cells (A-cells) emphasizes the close relationships between these two cell types, and it further supports the hypothesis of glicentin as a glucagon precursor. DIABETES 29:156–158, February 1980.

Recently, the immunocytochemistry of the pancreatic and gastric A-cell and of the intestinal L-cell has been extensively investigated in our laboratory.[1–5] Three types of antisera were used: specific (C-terminal) and nonspecific (N-terminal) antiglucagon sera and the R64 antiglicentin serum. This latter, which does not bind glucagon, was raised against the purified gut GLI of 100 amino acids called glicentin.[3,4] Glicentin contains the full amino acid composition and the entire sequence of glucagon[6,7] and, except for the inversion of two amino acids, the same C-terminal peptide as the proglucagon fragment of Tager and Steiner.[8]

With these antisera, it was demonstrated that the A-cell of the endocrine pancreas and of the oxyntic mucosa contains the immunoreactive sites that bind C- and N-terminally directed antiglucagon sera and the glicentin immunoreactive site, whereas the L-cell of the postduodenal mucosa reacts solely with N-terminal antiglucagon and with the antiglicentin sera.[4,5] These immunocytochemical and biochemical studies lead to the following hypothesis: (1) glicentin might be a precursor of glucagon biosynthesis; (2) the A-cells and L-cells differ in the degree to which they shorten a common primary gene product. In the A-cell, the biosynthetic pathway of glucagon would expose the 23–29 immunodeterminant, whereas in the L-cell this sequence would be masked by maintaining the C-terminal prolongation present in the proglucagon fragment. In support of this hypothesis, Tager and Markese[9] have been able to show recently that enzymatic digestion of two large glucagon-like substances isolated from rabbit intestine resulted in tryptic peptides immunologically and electrophoretically indistinguishable from those of glucagon. In this work, we performed similar enzymatic treatment on sections of ileal mucosa, and we provide morphologic evidence that such treatment leads to the unmasking of a C-terminal glucagon immunodeterminant in the L-cells.

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MATERIALS AND METHODS

Segments of dog ileum were fixed in Bouin's fluid, washed overnight in tap water, dehydrated in alcohol, and embedded in paraffin. Fragments of the same tissue were fixed in 4% phosphate-buffered glutaraldehyde, dehydrated, and embedded in Epon 812 without osmification.

The indirect immunofluorescence method of Coons et al.[10] was applied to rehydrated sections of paraffin-embedded ileum and to semithin sections (1 μm thick) pretreated to remove resin.[11]

The following antisera were used: rabbit antiglicentin serum (R64 provided by Dr. A. J. Moody, Novo, Copenhagen), rabbit antiglucagon serum 05Y, which reacts with intestinal L-cells and is considered a cross-reactive antiglucagon serum, and the specific rabbit antiglucagon serum 15K, which only reacts with pancreatic and gastric A-cells.[1] (05Y and 15K antisera were gifts of Dr. R. H. Unger, Dallas, Texas.) Sections were incubated for 2 h at room temperature in a moist chamber with the primary antiserum, repeatedly washed with PBS, and incubated for an additional hour with sheep antirabbit IgG serum coupled to FITC. The sections were then stained with 0.01% Evans blue and were observed in a Leitz Orthoplan fluorescence microscope equipped with a Pleomopak illuminator.

Enzymatic digestion. The enzymatic treatment was per-

formed after rehydration of paraffin-embedded tissue or after resin removal of Epon-embedded sections. The following schedule was used: first, a solution of 0.06% trypsin in 0.5 M Tris buffer was applied to the sections, and they were left at room temperature for 30 min. After rinsing with the same buffer, carboxypeptidase B (20 μg/ml in PBS) was applied for 30 min. The sections were then washed with Tris buffer, transferred to PBS, and finally processed for indirect immunofluorescence.

Controls of the specificity were carried out by absorbing each antiserum with glicentin, glucagon, or the related peptides VIP, GIP, and secretin (100 μg/ml of undiluted antiserum). The antigen-antibody mixture was left for 1 h at room temperature or overnight at 4°C before being applied to the sections.

RESULTS

Sections of paraffin- or Epon-embedded ileal mucosa, incubated with antiglicentin serum or with nonspecific N-terminally directed antiglucagon sera, showed the same population of cells stained by the two antisera. No immunoreactive cells were detected by the specific (C-terminally directed) antiglucagon serum (Figure 1A). If exposure to the specific antiglucagon serum was preceded by enzymatic treatment with trypsin and carboxypeptidase B, however, numerous cells in the glands and in the villi appeared brightly fluorescent (Figure 1B). These glucagon-immunoreactive cells could be easily identified as L-cells on the consecutive semithin section exposed to the antiglicentin serum without enzymatic treatment (compare Figures 1B and 1C). No fluorescent cells were found when the specific antiglucagon serum was preabsorbed with glucagon, whereas glicentin, VIP, GIP, and secretin did not quench the immunofluorescence. Treatment with enzymes did not appreciably alter immunofluorescence after exposure to antiglicentin or N-terminal antiglucagon sera.

DISCUSSION

The data presented in this paper clearly demonstrate that the intestinal L-cell contains antigenic sites that bind a specific antiglucagon serum. In this respect the L-cell does not differ from the glucagon-containing cell (A-cell) of the endocrine pancreas and the oxyntic mucosa, since each cell type contains the immunodeterminants of antiglicentin and of N-terminally and C-terminally directed antiglucagon sera. However, the L-cell does differ from the A-cell in that the C-terminal sequence of glucagon is usually not available for the corresponding antibody. For this reason, the L-cell cannot be identified by specific antiglucagon sera under conditions that allow the same cell to be easily detected by nonspecific antiglucagon sera and by the antiglicentin serum.[4,5]

This is reflected biochemically by the fact that the family of glucagon-like peptides extracted from the intestine, including glicentin, easily reacts with nonspecific antiglucagon sera but not with specific antiglucagon sera.[1,3,9,12] After limited enzymatic digestion, these peptides have been reported to yield tryptic fragments indistinguishable from the 18–29 sequence of glucagon.[7,9]

These findings perfectly correlate with the present morphologic demonstration that a similar enzymatic digestion on fixed tissue provokes the unmasking of antigenic sites

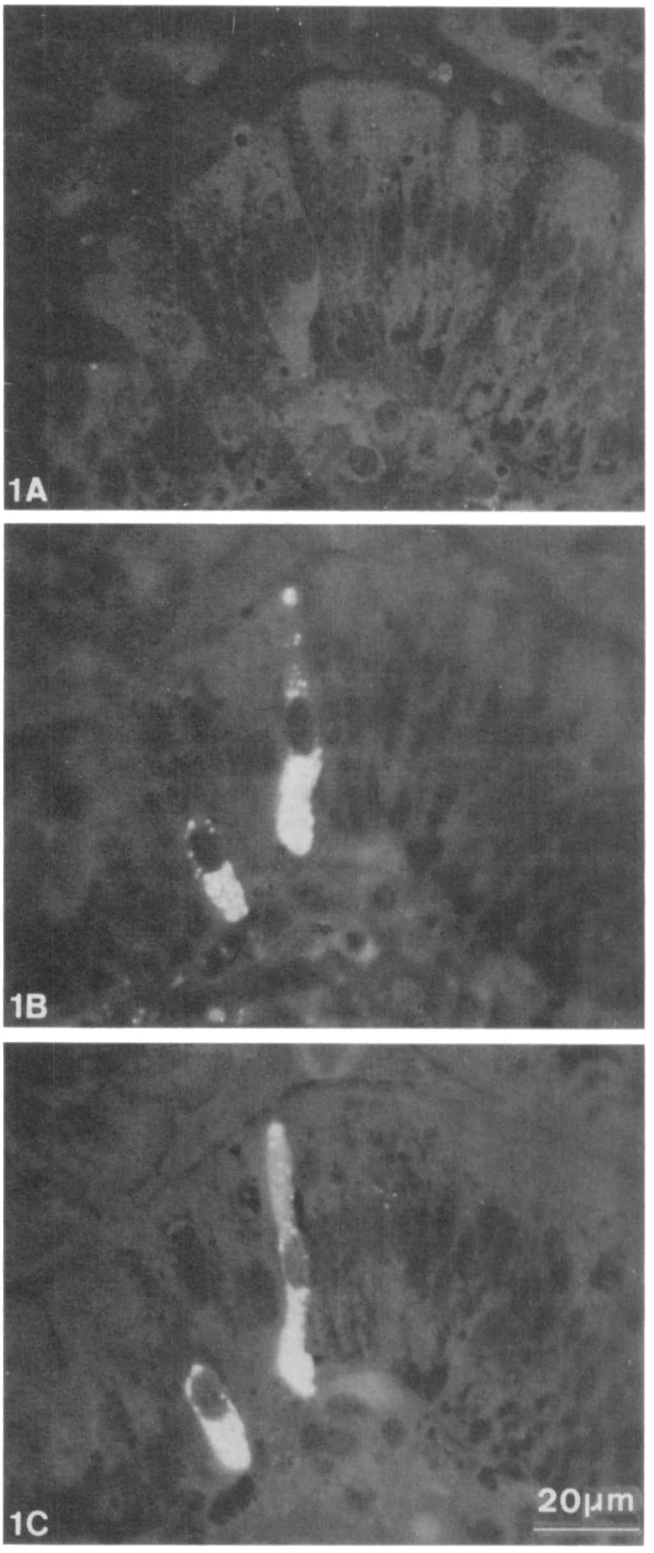

FIGURE 1. Consecutive semithin sections of dog ileum mucosa showing that glucagon C-terminal immunodeterminant is present in the L-cells and can be revealed by enzymatic digestion.

(A) Section not exposed to trypsin and carboxypeptidase B and incubated with specific antiglucagon serum: no fluorescent cells are revealed.

(B) Section exposed to trypsin and carboxypeptidase B and incubated with specific antiglucagon serum: immunofluorescent cells are present.

(C) Section not exposed to trypsin and carboxypeptidase B and incubated with antiglicentin serum: glicentin immunoreactive cells are the same as those positive to glucagon shown in B.

reacting with a specific antiglucagon serum in the same cell that contains glicentin[4,5] or gut GLIs.[13–16]

From this work, it can be concluded that the L-cell shares the same antigenic sites with the glucagon-containing cell (A-cell) and that, in the L-cell, the C-terminal sequence of glucagon is usually concealed but can be exposed by enzymatic digestion. The possible physiologic importance of this finding remains to be established.

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REFERENCES

[1] Baetens, D., Rufener, C., Srikant, B. C., Dobbs, R., Unger, R., and Orci, L.: Identification of glucagon-producing cells (A-cells) in dog gastric mucosa. J. Cell Biol. *69*:455–64, 1976.

[2] Moody, A. J., Frandsen, E. K., Jacobsen, H., Sundby, F., and Orci, L.: The structural and immunologic relationship between gut GLIs and glucagon. Metabolism *25* (Suppl. 1):1336–38, 1976.

[3] Moody, A. J., Jacobsen, H., Sundby, F., Frandsen, E. K., Baetens, D., and Orci, L.: Heterogeneity of gut glucagon-like immunoreactivity (GLI). *In* Glucagon: Its Role in Physiology and Clinical Medicine. Foà, P. P., Bajaj, J. S., and Foà, N. L., Eds. Berlin, Springer Verlag, 1977, pp. 129–35.

[4] Ravazzola, M., Siperstein, A., Moody, A. J., Sundby, F., Jacobsen, H., and Orci, L.: Glicentin immunoreactive cells: their relationship with glucagon producing cells. Endocrinology *105*:499–508, 1979.

[5] Ravazzola, M., Siperstein, A., Moody, A. J., Sundby, F., Jacobsen, H., and Orci, L.: Glicentin. A precursor of glucagon? Life Sci. *25*:287–90, 1979.

[6] Jacobsen, H., Demandt, A., Moody, A. J., and Sundby, F.: Sequence analysis of porcine gut GLI-1. Biochim. Biophys. Acta *493*:452–59, 1977.

[7] Holst, J. J., and Hagerup, M.: Evidence that the glicentin molecule contains the entire glucagon sequence. 10th Congr. IDF, Vienna, 1979. Amsterdam, Excerpta Medica. Abstract.

[8] Tager, H. S., and Steiner, D. F.: Isolation of a glucagon-containing peptide: primary structure of a possible fragment of proglucagon. Proc. Natl. Acad. Sci. USA *70*:2321–25, 1973.

[9] Tager, H. S., and Markese, J.: Intestinal and pancreatic glucagon-like peptides. Evidence for higher molecular weight forms. J. Biol. Chem. *254*:2229–33, 1979.

[10] Coons, A. H., Leduc, E. H., and 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*:49–63, 1955.

[11] Maxwell, M. H.: Two rapid and simple methods used for the removal of resin from 1.0 μm thick epoxy sections. J. Microsc. *112*:253–55, 1978.

[12] Heding, L. G., Frandsen, E. K., and Jacobsen, H.: Structure-function relationship: immunologic. Matabolism *25* (Suppl. 1). 1327–29, 1976.

[13] Polak, J. M., Bloom, S., Coulling, I., and Pearse, A. G. E.: Immunofluorescent localization of enteroglucagon cells in the gastrointestinal tract of the dog. Gut *12*:311–18, 1971.

[14] Larsson, L.-I., Holst, J., Håkanson, R., and Sundler, F.: Distribution and properties of glucagon immunoreactivity in the digestive tract of various mammals: an immunohistochemical and immunochemical study. Histochemistry *44*:281–90, 1975.

[15] Grimelius, L., Capella, C., Buffa, R., Polak, J. M., Pearse, A. G. E., and Solcia, E.: Cytochemical and ultrastructural differentiation of enteroglucagon and pancreatic type glucagon cells of the gastrointestinal tract. Virchows Arch. (Cell Pathol.) *20*:217–28, 1976.

[16] Ito, S., and Kobayashi, S.: Immunocytochemical demonstration of glucagon- and GLI-containing cells in the canine gut and pancreas. Arch. Histol. Jpn. *39*:193–202, 1976.