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Immunocytochemical localization of prosomatostatin fragments in maturing and mature secretory granules of pancreatic and gastrointestinal D cells

(staphylococcal protein A–gold/prohormone conversion/somatostatin/pancreas/gut)

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ABSTRACT Pancreatic and gastrointestinal D cells were examined by immunocytochemistry using antisera against somatostatin-28 (SS28) and its NH_2-terminal fragment SS28-(1–12), followed by the staphylococcal protein A–gold (pAg) complex. In pancreatic and gastric D cells incubated with antiserum against SS28-(1–12) the gold particles produced intense staining of the mature secretory granules but weaker staining of the immature granules associated with the Golgi area, whereas after SS28 antiserum treatment the particles accumulated selectively over the population of immature secretory granules. In intestinal D cells not only SS28-(1–12) but also SS28 antiserum produced an intense gold staining over the mature δ granules. These observations show that the relative amounts of immunoreactive sites related to SS28 and its cleavage product SS28-(1–12) in maturing and mature secretory granules are different in pancreatic, gastric, and intestinal D cells.

The tetradecapeptide somatostatin (SS14), originally isolated from the hypothalamus (1), was subsequently found to possess a wide but characteristic anatomical distribution. By using radioimmunoassay or immunocytochemistry, somatostatin immunoreactivity has been localized throughout the nervous system, pancreas, and gut (2–11). The presence in these tissues of immunoreactive material of a molecular size greater than that of the tetradecapeptide raised the possibility that somatostatin may be synthesized, like other secretory polypeptides, as part of a larger precursor molecule (5, 11–16). This has been confirmed by recent studies using pulse–chase-labeled islets of Langerhans (17, 18) and cell-free translation systems (19–23) as well as determining the sequence of the cDNA of the somatostatin gene (24). Among the larger molecular forms of somatostatin, a 28-amino-acid polypeptide, somatostatin-28 (SS28), characterized as an NH_2-terminally extended form of SS14, has been proposed (25–29) as an immediate precursor from which somatostatin could originate by enzymatic cleavage at the dibasic structure Arg-Lys preceding the NH_2-terminus of SS14. To see whether the anatomical location would affect the intracellular processing of prosomatostatin we have performed an immunocytochemical study on the pancreatic and gastrointestinal D cells by using two antisera, one directed against SS28 and the other against the dodecapeptide cleavage product SS28-(1–12) recently characterized in murine pancreas (29).

MATERIALS AND METHODS

Tissue. Human pancreatic and gastrointestinal tissues obtained during surgery for treatment of diseases of the pancreas and of the gastrointestinal tract were fixed in either Bouin's fluid or 2.5% (wt/vol) glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Material fixed with Bouin's fluid was dehydrated and embedded in paraffin for light microscopy. Glutaraldehyde-fixed tissue was prepared for electron microscopy without the postosmication step. The pancreas and gastrointestinal tract from adult rats (250–300 g) were processed in the same way. Pancreas from human fetus (12 cm crown to rump) was also studied. This material belonged to a series of previously studied fetuses fixed by vascular perfusion and processed as detailed elsewhere (30).

Immunofluorescence. Sections of paraffin- or Epon-embedded material were processed by the indirect immunofluorescence method after removal of the embedding medium (31). After exposure to the primary antiserum for 2 hr at room temperature, sections were washed and allowed to react with sheep anti-rabbit IgG coupled to fluorescein isothiocyanate. Sections were subsequently counterstained with Evans blue (0.01%) and examined with a Leitz Orthoplan microscope equipped for fluorescence.

Immunoelectron Microscopy. The staphylococcal protein A–gold (pAg) technique was applied (32). Briefly, thin sections collected on nickel grids were incubated overnight at 4°C with the antisera, washed in phosphate-buffered saline, and exposed to a solution of pAg (1:100). Sections of osmium tetroxide-treated tissue were etched 10 min with 5% (vol/vol) H_2O_2 before application of the antisera. Sections were counterstained with uranyl acetate and lead citrate and examined with a Philips EM 301 electron microscope.

Controls. Specificity controls were performed by adsorbing each antiserum with SS14, SS28, or SS28-(1–12).

Antisera. S309 JA3-81 was produced against SS28-Tyr conjugated to bovine serum albumin by using bis-diazotized benzidine. The antigenic determinant is located in segment 1–14 of SS28. This antiserum does not read SS14, as tested in standard liquid-phase radioimmunoassay. The percentage of crossreactivity with SS28-(1–12) was 10% on a molar basis. For immunocytochemistry, S309 was adsorbed with an excess of SS28-(1–12) (1 mg/ml) and diluted 1:400 before being applied to the sections.

S320-21-10-81 was produced against Tyr-SS28-(1–12) conjugated to bovine serum albumin by using bis-diazotized benzidine as the coupling agent. The antigenic determinant is SS28-(5–12). By radioimmunoassay, crossreactivity was absent with SS14 and minimal (0.01%) with SS28. This antiserum was diluted 1:1,600.

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Abbreviations: SS28, somatostatin-28; SS14, somatostatin-14; pAg, staphylococcal protein A–gold.

RESULTS

Pancreatic D Cells. In light microscopy, both somatostatin antisera yielded specific immunofluorescence on the D cells. However, whereas SS28-(1–12) antiserum produced a diffuse cytoplasmic staining, anti-SS28 elicited spot fluorescence in the perinuclear region of the D cells (not shown). In electron microscopy the D cells incubated with somatostatin antisera fol-

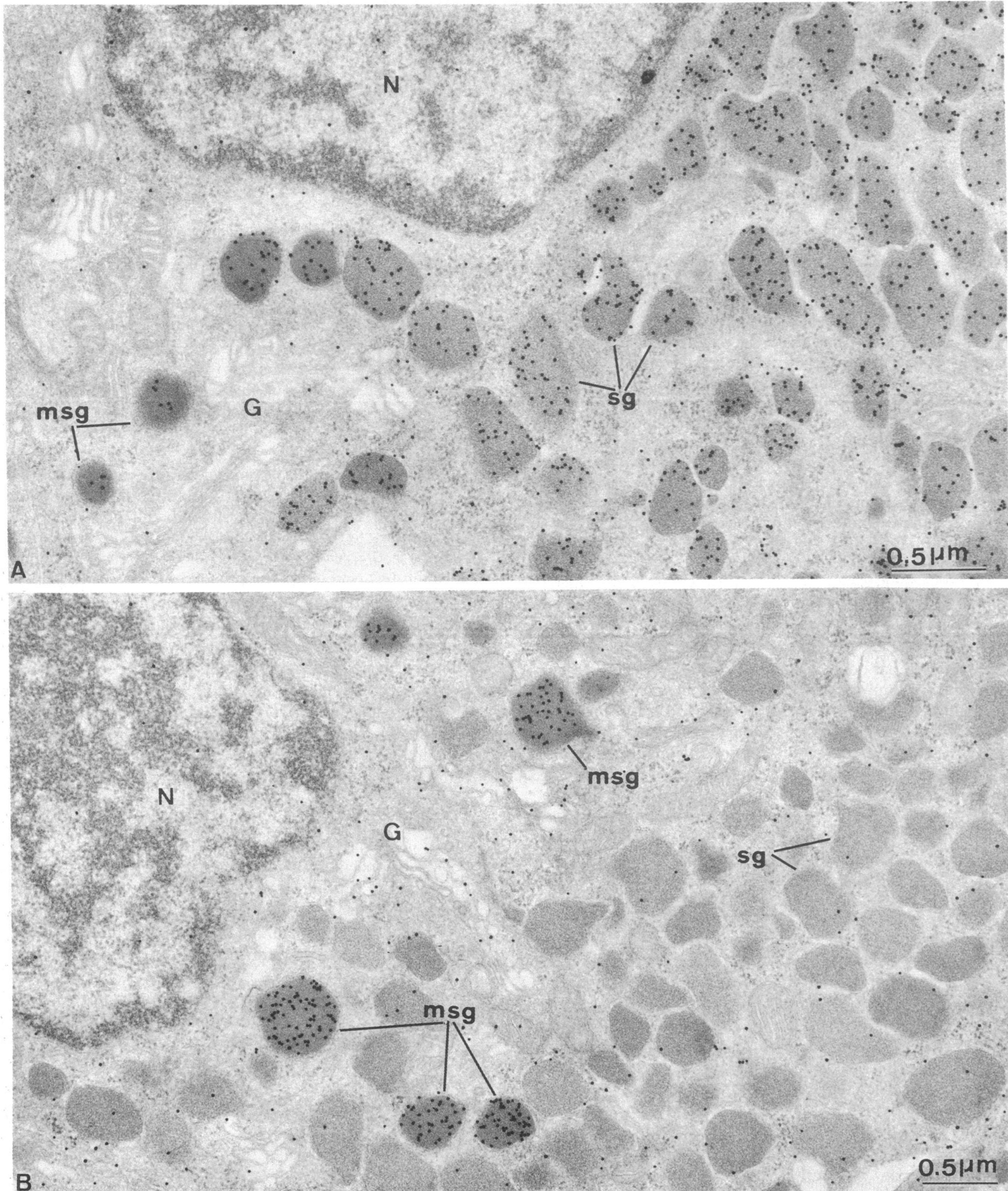

FIG. 1. Thin sections of pancreatic D cells from human fetus. (*A*) After treatment with SS28-(1–12) antiserum and the pAg complex the mature secretory granules (sg) appear intensely stained with gold particles, while maturing secretory granules (msg) in the Golgi area (G) show a weaker degree of staining. (×32,000.) (*B*) Immunostaining with SS28 antiserum and the pAg complex reveals a reverse pattern consisting of high levels of immunoreactivity in maturing secretory granules (msg) and a very low degree of staining over the bulk of mature δ granules (sg). N, nucleus. (×27,000.)

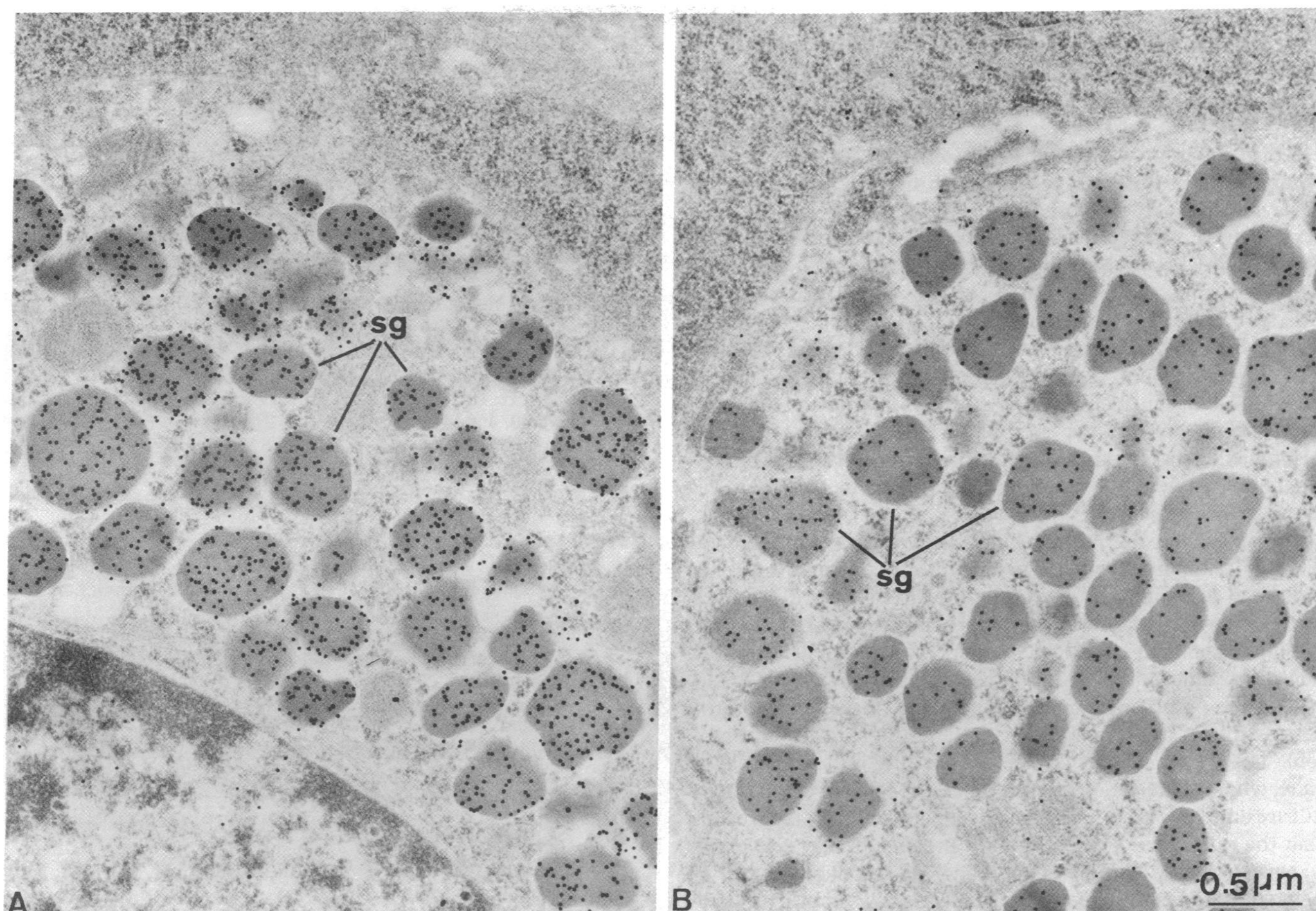

FIG. 2. Duodenal mucosa of human adult. Thin sections of D cells after incubation with SS28-(1–12) antiserum (*A*) and with SS28 antiserum (*B*) followed by the pAg complex. Each antiserum produced intense staining by gold particles over the mature secretory granules (sg), indicating high levels of SS28-(1–12) and SS28 immunoreactive sites in this compartment. (×25,000.)

lowed by the pAg complex appeared specifically stained by gold particles. The immunostaining was abolished by adsorbing each antiserum with the homologous antigen but not by adsorption of anti-SS28-(1–12) with SS28 or of SS28 antiserum with the 1–12 fragment. No crossreactivity with SS14 was found.

With anti-SS28-(1–12) the gold particles produced intense staining of the mature secretory granules and weaker staining of the population of maturing secretory granules defined by a highly electron-dense core, a coated segment on their membrane, and their association with the Golgi area (Fig. 1*A*). With anti-SS28, the gold particles accumulated selectively over the immature secretory granules of the Golgi area (Fig. 1*B*), accounting for the spot fluorescence observed in light microscopy, whereas only occasional gold particles were observed over the bulk of mature secretory granules. This staining pattern was found in both rat and human adult and fetal pancreatic D cells.

Gastrointestinal Tract. Gastric D cells of the oxyntic and pyloric mucosa immunostained with somatostatin antisera and the pAg complex revealed the same staining pattern as the pancreatic D cells. In the intestinal mucosa the secretory granules of the D cells were also intensely stained by gold particles after addition of SS28-(1–12) antiserum (Fig. 2*A*), but, at variance with the pancreatic and gastric pattern, maturing and mature secretory granules showed equally strong immunoreactivity to anti-SS28 (Fig. 2*B*).

DISCUSSION

The presence of larger molecular forms of somatostatin, in addition to the tetradecapeptide, has been reported in somatostatin-containing tissues, and an NH_2-terminally extended form, SS28, has been isolated and proposed as a precursor that by enzymatic cleavage may yield the tetradecapeptide and the NH_2-terminal fragment SS28-(1–12).

Indirect evidence for this and for alternative processing patterns was recently provided by data showing large amounts of SS14 and SS28-(1–12) in pancreatic and hypothalamic tissue (29) but a predominance of SS28-like immunoreactivity in extracts of intestinal mucosa (33), suggesting that somatostatin precursors may be processed by different mechanisms in different tissues. This possibility has now been explored by an immunocytochemical method, the pAg technique, that permits the direct correlation of the amount of a given antigenic site with a given secretory compartment. From the results presented here it appears clear that antigenic sites related to the putative precursor SS28 and to the cleavage product SS28-(1–12) in this study are differently distributed in the D cells, depending on their anatomical location. In pancreatic and gastric D cells stained with SS28 antiserum, a selective, intense staining by gold particles was found over the maturing secretory granules but only very weak staining over the mature granules. This was paralleled by a modest but sizeable amount of cleavage product SS28-(1–12) antigenic sites over the maturing secretory granules and a very high concentration of these immunoreactive sites over the mature granules. This suggests that, in pancreatic and gastric D cells, somatostatin precursors undergo a rapid conversion process that starts as far back as the Golgi region, where the granules are formed, and is virtually completed in the mature granules. In the intestinal mucosa the staining pattern of

the D cells was basically different, because a comparable high density of SS28 antigenic sites was found over the maturing and mature secretory granules. That such a difference could be an artefact due to the fixation and embedding of the tissue is unlikely because the same pattern was reproduced in tissues processed in different ways. Therefore, one can assume that the staining patterns observed reflect major differences in the precursor-to-product ratios within the maturing and mature secretory granules of the pancreatic and gastric D cells and of the intestinal D cells. When using specific antisera directed against the NH_2-terminal portion of SS14 we were unable to detect the tetradecapeptide in pancreatic islets. The tetradecapeptide SS14 has been characterized in rat pancreas and shown to constitute the major form of somatostatin-like immunoreactivity in that tissue (34). The inability to visualize SS14 in pancreas when using specific antisera may be related to alteration of its NH_2 terminus during fixation, or it may be that the NH_2 terminus is bound to the vesicular membrane or to a specific carrier inside the secretory granule.

In conclusion, the immunocytochemical data presented in this study support the idea that somatostatin precursors can be processed to a different degree in different tissues. This adds to the growing evidence that different posttranslational processing of a common hormonal precursor can generate different peptide products in different tissues. A classical example is the case of the proopiomelanocortin molecule, for which the processing differs in the nervous tissue and the anterior pituitary (35). These two ectodermal tissues are different embryologically, whereas the pancreatic islets and the mucosa of the upper gut are embryologically similar. The present work demonstrates that the difference in processing of precursors may also apply to closely related tissues such as pancreas and duodenum. Recent studies performed with brain tissue show that the proportions of peptides derived from prosomatostatin vary in different brain regions (36).

The observation of antigenic determinants in the mature secretory granules before exocytosis strongly suggests that SS28-(1–12) is a major secretory product of the D cells of the pancreas and stomach.

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