Immuno-Fluorescent Reactivity to Anti-Somatostatin in the Gastro-Intestinal Mucosa of the Dog

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Received: May 17, 1975, and in revised form: May 23, 1975

Summary. Evidence is presented that somatostatin-containing cells are present in the gastro-intestinal tract of the dog. Thus immuno-fluorescent cells were detected by the use of antiserum to cyclic somatostatin. These cells were mainly encountered in the antral mucosa and in the neighbourhood of gastrin-producing cells. No cross reaction was observed between gastrin and somatostatin. It is suggested that locally produced somatostatin controls gastrin

secretion, and, more generally, that somatostatin-containing cells, multifocally distributed, modulates secretion of a large number of glands.

Key words: Immuno-fluorescence, gastro-intestinal tract, somatostatin, gastrin.

Somatostatin, a growth hormone release-inhibiting factor (or GIF) has been isolated from the hypothalamus [1]. In addition to its action on growth hormone release, somatostatin has been demonstrated to inhibit the release of insulin, glucagon, gut glucagon, thyroid stimulating hormone and gastrin [2–16]. Antibodies to somatostatin allowed immunohistochemical studies in the hypothalamus [17–19] and the endocrine pancreas [20, 21]. We report here the presence of immunohistochemically positive cells to anti-somatostatin serum in the gastro-intestinal tract of the dog¹.

Material and Methods

Tissue Preparation

The different portions of the GI tract of dogs were fixed in Bouin's solution (2.2% picric acid, 10% formaldehyde and 5% acetic acid), dehydrated, and embedded in paraffin. Five microns thick sections were mounted on albumin-covered slides, rehydrated after removal of paraffin and processed for immunohistochemistry.

Antisera

Cyclic somatostatin was conjugated to human serum albumin by means of glutaraldehyde or diamino-benzidine and emulsified in Freund's com-

plete adjuvant [19]. Rabbits were immunized by the injection of 1100 µg of somatostatin per animal, divided in three doses administered at three weeks intervals [21]. For immuno-fluorescence the antiserum was diluted 1:200 in phosphate buffered saline (PBS). Antibodies to synthetic human gastrin I (SHG 2–17, Imperial Chemical Ind. Ltd) raised in rabbits were a generous gift from Dr. W. Gepts (Vrije Universiteit, Brussels, Belgium) [23]. Fluorescein isothiocyanate (FITC) conjugated to sheep antirabbit immunoglobulin was obtained commercially (Pasteur Institute, Paris).

Controls

The following controls were used:

- a) anti-somatostatin serum mixed with an excess of synthetic somatostatin (200 µg/ml).
- b) anti-somatostatin serum mixed with an excess of synthetic gastrin (50 µg/ml).
- c) anti-gastrin serum mixed with an excess of synthetic gastrin (50 µg/ml).
- d) anti-gastrin serum mixed with an excess of synthetic somatostatin (200 µg/ml).
- e) normal rabbit serum.
- f) FITC alone.

Immuno-Fluorescence

The indirect immuno-fluorescence technique was applied [24]. Sections were incubated with the first antiserum (anti-somatostatin, anti-gastrin or the con-

¹ While this manuscript was in preparation, Arimura and coworkers have reported on the presence of somatostatin in high concentrations in gastric and pancreatic extracts of the rat [22].

Fig. 1. Two serial sections from dog antral mucosa. a) Incubation with anti-somatostatin serum. Four fluorescent cells are seen in the glands. b) Incubation with anti-somatostatin serum adsorbed with an excess of synthetic somatostatin (control a). Fluorescence is completely abolished in the cells
× 400

trols) at room temperature for one hour, rinsed with PBS, incubated with FITC for one hour, rinsed again with PBS, counterstained with Evans'Blue (0.01%) for 5 min, and mounted in glycerin-PBS. The sections were observed in a Zeiss Ultraphot microscope equipped with a III RS condenser.

Results

After incubation with anti-somatostatin serum, immuno-fluorescent cells were found along the entire gastro-intestinal tract. They were particularly abundant, however, in the antral mucosa, in the neighbourhood of gastrin-producing cells (Fig. 1a). In view of the prevalence of somatostatin-containing cells in regions which are also abundant in gastrin-producing cells, we tested the sections for a possible cross-reaction between gastrin and somatostatin. The anti-somatostatin immuno-fluorescence was eliminated by prior incubation of the serum with synthetic somatostatin (control a; Fig. 1b), but was not suppressed by mixing the anti-somatostatin serum with

Fig. 2. Two serial sections from dog antral mucosa. a) Incubation with anti-somatostatin serum. Several fluorescent cells are scattered in the glandular epithelium. b) Incubation with anti-gastrin serum. Fluorescent cells are more numerous. Note that all the cells exhibiting fluorescence in a) are different from the fluorescent cells in b)

synthetic gastrin (control b). Conversely, gastrin immuno-fluorescence was abolished after adsorption of the specific serum with synthetic gastrin (control c), but remained unaffected by adsorption with synthetic somatostatin (control d). Incubation of the sections with normal rabbit serum followed by FITC, or with FITC alone failed to produce any fluorescence (controls e and f). Finally, by studying serial sections, it was shown that cells with anti-somatostatin immuno-fluorescence were different from those with anti-gastrin immuno-fluorescence (Fig. 2a, b).

Discussion

The present results suggest that somatostatin is present in the GI tract of the dog. This statement is made with the reservation that immuno-fluorescence does not allow a determination of the precise structure of the detected antigen, and hence cannot completely exclude a possible cross-reaction with a closely related polypeptide. The presence of somatostatin-containing cells in the GI tract raises the question of their function.

nal significance. The detection of somatostatin-containing cells in pancreatic islets [20, 21], and the demonstration of an inhibitory effect of somatostatin upon insulin and glucagon release [2–14] had led to the suggestion that locally produced somatostatin might control pancreatic endocrine function [20, 21]. Since somatostatin inhibits gastrin secretion [16] and since somatostatin-containing cells lie in close neighbourhood to gastrin-producing cells, we suggest that locally produced somatostatin might also control gastrin secretion. More generally, in view of the influence of somatostatin on the release of various secretion products [2–16, 25–28], and of the multifocal distribution of somatostatin-containing cells, we suggest that somatostatin modulates secretion in a large number of organs, yet affecting selectively those secretory cells neighbouring its site of release. This would then provide an explanation for our finding that somatostatin-containing cells are distributed throughout the entire GI tract, where they could influence directly a variety of secretory cells [14, 16, 25–28]. The challenge that now confronts us is to characterize at the ultrastructural level the cell type corresponding to the somatostatin-containing cell. The pancreatic and gastro-intestinal D-cell (for review see 29) is the best candidate for being the somatostatin-containing cell. In this respect we have recently found that the pancreas of the pigeon, which is particularly rich in D-cells (A_1 -cells) [30] has particularly numerous anti-somatostatin immuno-fluorescent cells [3].

Acknowledgments. We are grateful to Dr. J. Rivier and Dr. R. Guillemin for their generous gift of cyclic somatostatin. We are also very obliged to Dr. W. Gepts for providing synthetic gastrin and anti-gastrin serum. We wish to acknowledge the excellent technical assistance of Mrs Angelica Irribarra and Mr Max Baumann. This work was supported by grants No 3.808–1.72, 3.553.75, from the fonds National Suisse de la Recherche Scientifique, and by grant No 74–4–445–35 from the I.N.S.E.R.M. (France).

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Note Added in Proof: By using a peroxidase labelling technique, we were recently able to identify ultrastructurally the somatostatin-containing cell as the D-cell [32].