Cell Tiss. Res. 186, 181–185 (1978)

Cell and Tissue Research

Short Communication

Localisation of Somatostatin- and Gastrin-like Immunoreactivity in the Gastrointestinal Tract of *Ciona intestinalis* L.

H.A.R. Fritsch, S. Van Noorden* and A.G.E. Pearse*

Anatomisches Institut der Universität Kiel, Kiel, Germany (FRG)
* Department of Histochemistry, Royal Postgraduate Medical School, London, England

Summary. Somatostatin- and gastrin-like immunoreactivity has been found by immunofluorescence in cells of the stomach and intestinal epithelia of *Ciona intestinalis* L. The cells containing the peptide immunoreactive to mammalian anti-gastrin can be restained with the Grimelius' technique for argyrophilia.

Key words: Somatostatin – Gastrin – Gastrointestinal tract of *Ciona intestinalis* – Endocrine cells – Immunofluorescence.

Introduction

Although somatostatin (Somatotropin-release-inhibiting factor) was originally extracted from the hypothalamus (Brazeau et al., 1973; Arimura et al., 1975; Hökfelt et al., 1975), somatostatin-like immunoreactivity was demonstrated in the intestine and pancreas of mammals (Polak et al., 1975; Rufener et al., 1975; Dubois, 1975), as well as in the gastro-intestinal tract (GIT) of lower vertebrates (Van Noorden et al., 1977). Somatostatin is thought to be a potent inhibitor of gastrin release (Bloom et al., 1974) and seems to act directly on G-cells. As cross-reactions occur between antibodies to mammalian hormones and cells in the GIT of lower vertebrates (Van Noorden and Pearse, 1974; Östberg et al., 1975, 1976a, b) and invertebrates (Falkmer, 1972; Fritsch, 1976) and since endocrine-like cells could be demonstrated in tunicates by EM (Fritsch and Sprang, 1977), it seemed reasonable to test antisera to synthetic mammalian somatostatin and gastrin on the gastrointestinal tract of a protochordate, *Ciona intestinalis* L.

Materials and Methods

Ciona intestinalis were dredged in the western Baltic sea. The animals (30–40 mm) were often attached to *Laminaria saccharina* (Phaeophyceae). They were kept in aerated sea water (16‰) in plastic basins at 5° C.

Send offprint requests to: Dr. H.A.R. Fritsch, Anatomisches Institut der Universität Kiel, Neue Universität, Olshausenstr. 40–60, 23 Kiel, Federal Republic of Germany

0302-766X/78/0186/0181/$01.00

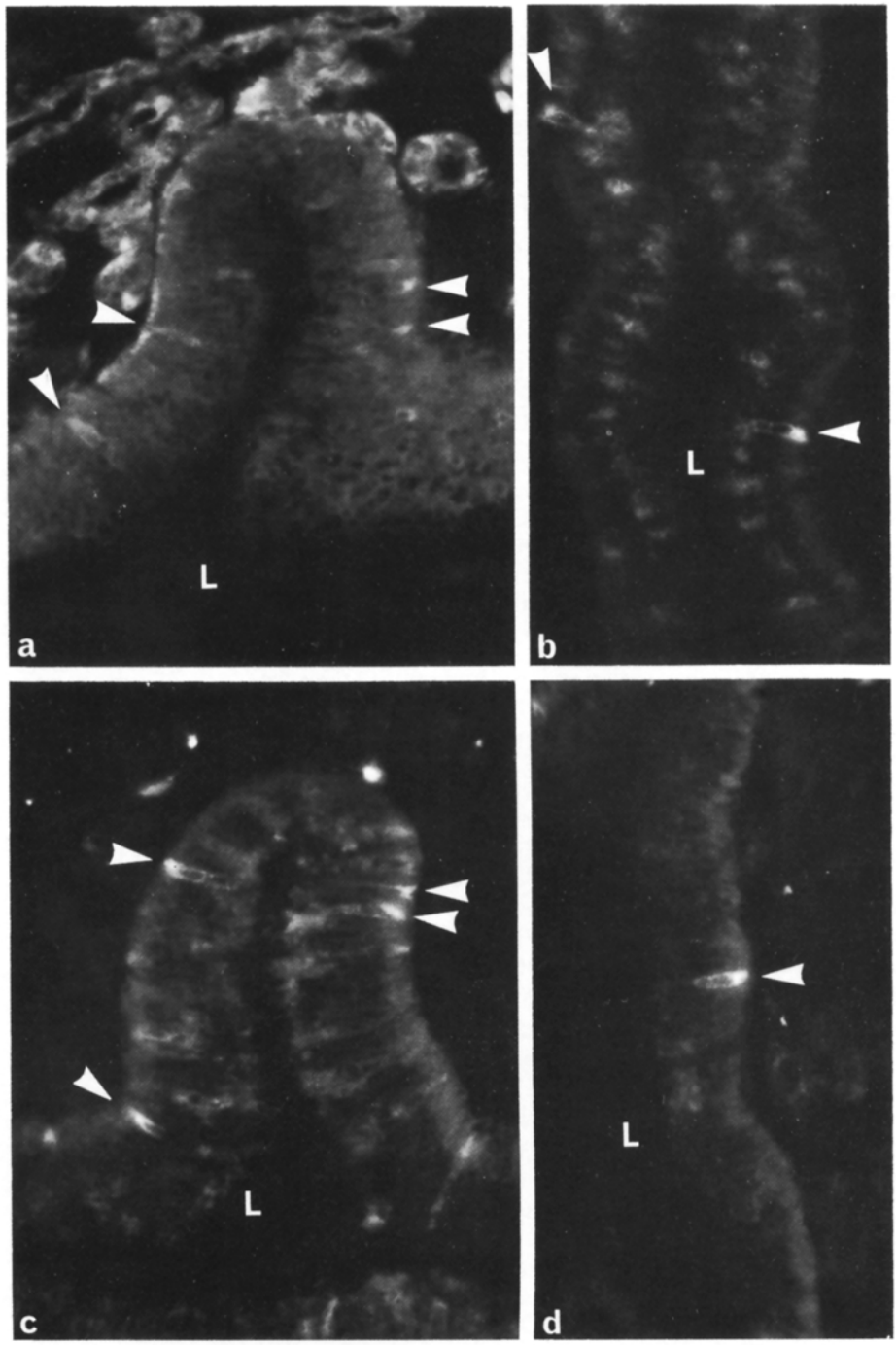

Fig. 1 a–d. Immunofluorescence photomicrographs showing the gastro-intestinal tract of *Ciona intestinalis* L. Somatostatin-like immunoreactivity is seen in cells of the epithelium of the stomach ridges **a** and the intestine **b** (arrows). Gastrin-like immunoreactivity is seen in cells of the stomach **c** and the intestine **d** (arrows). *L* lumen of the gastro-intestinal tract. × 570

Histological Methods. The oesophagus, stomach and intestine were dissected out. A short period of fixation (3 h) in Bouin's fluid or methanol-free formaldehyde (MFF) (Polak, Bussolati and Pearse, 1971), was followed by standard dehydration, embedding in paraplast and sectioning. Further specimens were freeze-dried and fixed in formaldehyde vapour at 60° C embedding in paraffin wax. Antibodies to synthetic cyclic somatostatin and synthetic human gastrin (1–17) (ICI) were produced in rabbits and tested by radioimmunoassay and by immunostaining in mammalian tissue (Polak et al., 1975). The indirect immunofluorescence technique (Coons et al., 1955) was applied to sections using rabbit anti-somatostatin or rabbit anti-gastrin as the first layer and fluorescein-conjugated goat anti-rabbit globulin as the second layer. Control staining was carried out with anti-somatostatin, which had been pre-absorbed with either somatostatin or gastrin and with anti-gastrin which had been pre-absorbed with either gastrin or somatostatin. Sections were mounted in buffered glycerine and examined in a Zeiss fluorescence microcope, Staining for argyrophilia was carried out by the Grimelius technique (Grimelius, 1968).

Results

In the stomach and the intestine of *Ciona intestinalis* large and apparently granular cells immunoreactive with antisera to somatostatin and gastrin could be seen. The strongest fluorescence was obtained in tissues fixed in Bouin's fluid. The cells are unevenly scattered, as single elements or in small groups within the epithelium. They were numerous in the stomach, but occurred more rarely in the upper and lower intestine. There were fewer somatostatin-immunoreactive than gastrin-immunoreactive cells. Although both immunoreactive cell types varied considerably in number from animal to animal, the gut epithelium contained at least a few in all cases examined. The cells normally rest upon the basal lamina of the simple columnar epithelium. In a correctly orientated longitudinal section they extend to the gut lumen and end with a filamentous projection. Anti-somatostatin serum which had been absorbed with gastrin gave positive immunofluorescence whereas no staining could be observed using anti-somatostatin which had been absorbed with somatostatin. Argyrophilic staining carried out on sections which had been previously immunostained for somatostatin showed that in no case were somatostatin-immunoreactive cells and argyrophil cells identical. In the same way anti-gastrin which had been absorbed with somatostatin gave positive immunofluorescence but no reaction was seen after pre-absorption with gastrin. Restaining for argyrophilia after immunofluorescence gave positive results for gastrin-like cells.

If the procedure was carried out in reverse order, staining first for argyrophilia and then removing the silver deposit with KCN and restaining for gastrin or somatostatin, it was found that the argyrophil cells were positively immunostained by anti-gastrin but not by anti-somatostatin. There were always several argyrophil cells, in addition to those which cross-reacted with anti-gastrin, present in every section.

Discussion

In gut endocrinology one of the most significant current developments is that which links gut and brain by the discovery of peptides common to both regions (Pearse, 1976; Pearse et al., 1977). This supports the theory that, with few exceptions, the

probable origin of peptide hormone producing cells is from neurally-programmed cells in the ectoblast. The finding of somatostatin-like immunoreactivity in the epithelium of the stomach and intestine of *Ciona intestinalis* agrees well with the findings in mammals as well as with earlier results showing the presence of peptide-producing cells in lower vertebrates and invertebrates (Wilson and Falkmer, 1965; Mehrotra and Falkmer, 1969; Falkmer, 1972; Davidson et al., 1972; Falkmer et al., 1973; Van Noorden and Pearse, 1974; Fritsch et al., 1976; Fritsch and Sprang, 1977; Van Noorden et al., 1977; Falkmer et al., 1977).

Although somatostatin has been detected by radioimmunoassay (RIA) in extracts of the GIT of *Ciona intestinalis* this is the first demonstration by histochemical techniques of the location of the SRIF-like cells in this species. As these cells cannot be restained with the silver technique of Grimelius (1968) it is likely that the GIT of *Ciona intestinalis* produces more than one polypeptide hormone in different cells (Fritsch, 1976). This is supported by the finding of a second cell-type with a gastrin-like immunoreactivity in the stomach and the intestine. These cells are argyrophil and probably correspond to some at least of the argyrophil cells described earlier (Fritsch, 1976) and which are quite different from those found by Falkmer et al. (1977). Further investigation with a combination of electron microscopy and immunocytochemistry is necessary to clarify the situation. In addition the embryological source of the somatostatin-like and gastrin-like cells requires further attention as does the physiological role of the hormone-like substances in invertebrates. There is nevertheless no doubt that the peptides produced in invertebrates must share antigenic sites with their mammalian equivalents.

References

Arimura, A., Sato, H., DuPont, A., Nishi, N., Schally, A.V.: Abundance of immunoreactive GH-release inhibiting hormone in the stomach and pancreas of rat. Fed. Proc. **34,** 273 (1975)

Bloom, S.R., Mortimer, C.H., Thorner, M.G., Besser, G.M., Hall, R., Gomez-Pan, A., Roy, V.M., Russell, R.C.G., Coy, D.H., Kastin, A.J., Schally, A.V.: Inhibition of gastrin and gastric acid secretion by growth hormone release inhibiting hormone. Lancet 1974 **II,** 1106–1109

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., River, J., Guillemin, R.: Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science **179,** 77–79 (1973)

Coons, A.H., Leduc, E.H., Connolly, J.M.: Studies on antibody production. 1. Method for the histochemical demonstration of specific antibody and its application to the study of the hyperimmune rabbit. J. exp. Med. **102,** 49–59 (1955)

Davidson, J.K., Falkmer, S., Mehrotra, B.K., Wilson, S.: Insulin assays and light microscopical studies of digestive organs in protostomian and deuterostomian species and in coelenterates. Gen. comp. Endocr. **17,** 388–401 (1971)

Dubois, M.P.: Immunoreactive somatostatin is present in discrete cells of the endocrine pancreas. Proc. nat. Acad. Sci. (Wash.) **72,** 1340–1343 (1975)

Falkmer, S.: Insulin production in vertebrates and invertebrates. Gen. comp. Endocr. **3,** 184–191 (1972)

Falkmer, S., Elde, R.P., Hellerström, C., Petersson, B., Efendic, E., Fohlman, Siljevall, J.B.: Some phylogenetical aspects on the occurrence of somatostatin in the gastro-entero-pancreatic endocrine system. Arch. Histol. Jap., in press (1977)

Falkmer, S., Emdin, E., Havu, N., Lundgren, G., Marques, M., Östberg, Y., Steiner, D.M., Thomas, N.W.: Insulin in invertebrates and cyclostomes. Amer. Zool. **13,** 625–638 (1973)

Fritsch, H.A.R.: The occurrence of argyrophilic and argentaffin cells in the gut of *Ciona intestinalis* L. Cell Tiss. Res. **175,** 131–135 (1976)

Fritsch, H.A.R., Sprang, R.: On the ultrastructure of polypeptide hormone-producing cells in the gut of the Ascidian *Ciona intestinalis* L. and the Bivalve, *Mytilus edulis* L. Cell Tiss. Res. **177,** 407–413 (1977)

Fritsch, H.A.R., Van Noorden, S., Pearse, A.G.E.: Cytochemical and immunofluorescence investigations on insulin-like producing cells in the intestine of *Mytilus edulis* L. Cell Tiss. Res. **165,** 365–369 (1976)

Grimelius, L.: A silber nitrate stain for cells in human pancreatic islets. Acta Soc. Med. upsalien. **73,** 243–270 (1968)

Hökfelt, T., Efendic, S., Hellerström, C., Johannsson, C., Luft, O., Arimura, R.: Cellular localization of somatostatin in endocrine like cells and neurons of the rat with special reference to the A_1 cells of the pancreatic islets and to the hypothalamus. Acta endocr. (Suppl.) (Kbh.) **80,** 5–40 (1975)

Mehrotra, B.K., Falkmer, S.: Occurrence of insulin producing cells in the gastrointestinal tract of invertebrates. Diabetologia **4,** 244 (1968)

Östberg, Y.: The entero-insular endocrine organ in a cyclostome, *Myxine glutinosa.* Umeå Univ. Medical Diss., New Seri. No. 15 (1976)

Österberg, Y., Boquist, L., Van Noorden, S., Pearse, A.G.E.: On the origin of islet cells in a cyclostome, *Myxine glutinosa.* Gen. comp. Endocr. **28,** 228–246 (1976a)

Östberg, Y., Van Noorden, S., Pearse, A.G.E.: Cytochemical immunofluorescence and ultrastructural investigations on polypeptide hormone localization in the islet parenchyma and bile duct mucosa of a cyclostome, *Myxine glutinosa.* Gen. comp. Endocr. **25,** 274–291 (1975)

Pearse, A.G.E.: The diffuse neuroendocrine system and the APUD concept: related "endocrine" peptides in brain, intestine, pituitary, placenta, and anuran cutaneous glands. Med. Biol. **55,** 115–125 (1977)

Pearse, A.G.E., Polak, J.M., Bloom, S.R.: The newer gut hormones. Cellular sources, physiology, pathology and clinical aspects. Gastroendocr. **72,** 746–761 (1977)

Polak, J.M., Pearse, A.G.E., Grimelius, L., Bloom, S.R., Arimura, A.: Growth-hormone release inhibiting hormones (GH-RIH) in gastrointestinal and pancreatic D cells. Lancet **1975 I,** 1220–1222

Rufener, C., Dubois, M.P., Malaisse-Lagae, F., Orci, L.: Immunofluorescent reactivity to somatostatin in the gastrointestinal mucosa of the dog. Diabetologia **11,** 321–324 (1975)

Van Noorden, S., Östberg, Y., Pearse, A.G.E.: Localization of somatostatin-like immunoreactivity in the pancreatic islets of the hagfish, *Myxine glutinosa* and the lamprey, *Lampetra fluviatilis.* Cell Tiss. Res. **177,** 281–285 (1977)

Accepted October 4, 1977