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Single Cellular Origin of Somatostatin and Calcitonin in the Rat Thyroid Gland

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Summary. Immunostaining of thin serial paraffin sections has shown that somatostatin is present in the same parafollicular cells as calcitonin in the adult rat thyroid gland. The number of cells containing both peptides is much smaller than the number containing calcitonin but not somatostatin.

Introduction

The widespread localisation of somatostatin (somatotrophin release-inhibiting factor) is now well established. This cyclic tetradecapeptide was originally found in the hypothalamus (Brazeau et al., 1973) and has now been found in other parts of the central and peripheral nervous system (Hökfelt et al., 1975a; Brownstein et al., 1975), in the pancreatic islets (Luft et al., 1974), in the gastrointestinal tract (Polak et al., 1975a) and in the thyroid gland (Parsons et al., 1976).

Somatostatin has a powerful inhibitory effect on the secretion of many gut hormones (Bloom and Polak, 1977). Its short half-life and extensive distribution indicate that its action is probably paracrine (local) rather than endocrine (via the circulation).

It has been suggested that in the thyroid, where somatostatin has been found by immunostaining in the parafollicular cells (Hökfelt et al., 1975a; Parsons et al., 1976) its action is that of a local mediator of thyroid hormone secretion.

Previous work has failed to establish whether somatostatin is present in the same parafollicular cells as calcitonin or in different ones. The "one cell, one hormone" theory is fast breaking down with the recent discoveries of several "pairs" or hormones in single cell types such as enkephalin and gastrin in some antral G cells (Polak et al., 1977), substance P and 5-HT (Heitz et al., 1976), motilin and 5-HT (Polak et al., 1975b) and possibly somatostatin itself and gastrin in some pancreatic islets (Parsons et al., 1976). These considerations led us to investigate the precise localisation of somatostatin in the thyroid gland.

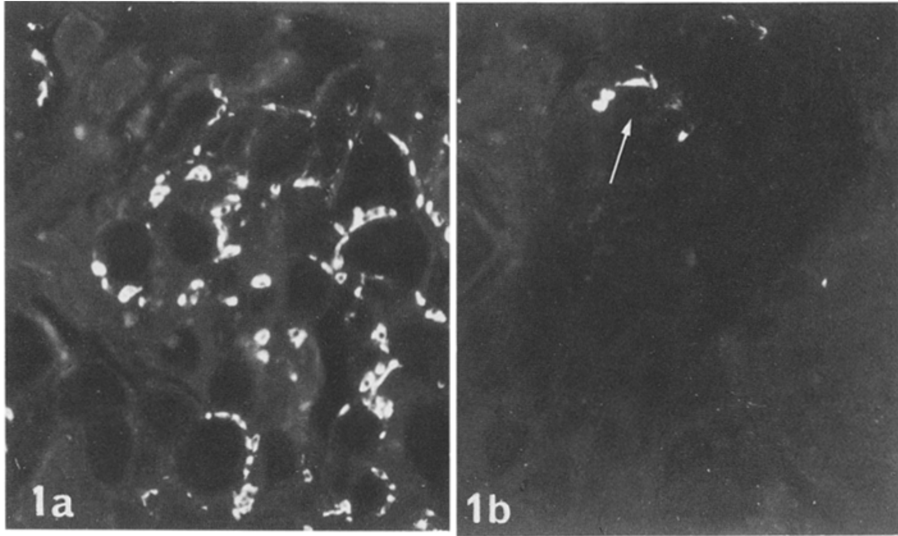

Fig. 1. a Rat thyroid gland. Immunofluorescent stain for calcitonin. (×140). **b** Serial section to 1a. Immunofluorescent stain for somatostatin. (×140). The number of somatostatin cells stained (arrow) is very small compared with the number of calcitonin-positive cells in Figure 1a

Material and Methods

Thyroid glands from 5 adult rats were freshly frozen in melting arcton (−156° C), freeze-dried, fixed in diethylpyrocarbonate vapour at 60° C (Pearse and Polak, 1975) and embedded in paraffin. Sections were cut serially through the glands in a cephalo-caudal plane at a thickness of 3 or 4 microns and were mounted on slides in groups of 10 to 20 sections. Alternate slides were stained for somatostatin and calcitonin using the Coons' indirect immunofluorescence technique (Coons et al., 1955) modified by using the primary antiserum at a high dilution and staining for 24 to 60 h at 4° C (Petrusz et al., 1975). Antisera to human calcitonin (Coombes et al., 1974) and to synthetic somatostatin (Polak et al., 1975b) were produced in rabbits. Three different antisera to somatostatin were used. The second layer antiserum was fluorescein-conjugated goat anti-rabbit globulin (Hyland) and was used at a dilution of 1/10 for one hour at room temperature.

In some cases antiserum to somatostatin was absorbed with somatostatin or with calcitonin before use and anti-calcitonin serum was absorbed similarly.

In many cases adjacent sections stained with the two antisera could be compared.

Results

Calcitonin immunofluorescence was found in parafollicular C cells in all sections within a central area about 200 microns from the periphery of the gland. The C cells were scattered widely through the sections but tended to be concentrated in a broad band across the middle of the gland.

Somatostatin-containing cells were revealed by all three antibodies and were limited to the surroundings of two or three follicles per gland. It was rare to find more than 5 or 6 somatostatin cells in any one section (Fig. 1a and

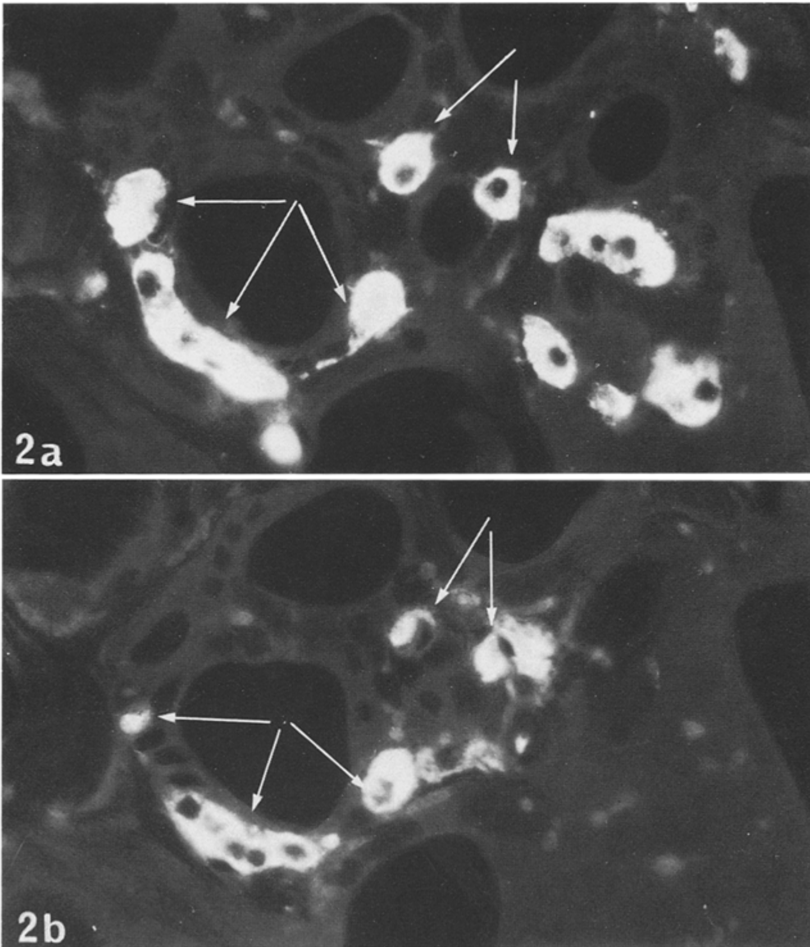

Fig. 2. a Rat thyroid gland. Immunofluorescent stain for calcitonin. (×508). **b** Serial section to 2a. Immunofluorescent stain for somatostatin. The arrows show some cells which are both somatostatin-positive and calcitonin-positive (×508)

b). In the small series investigated it was not possible to determine whether there was a specific topographical location within the gland for the somatostatin-containing cells. In any one gland there might be two somatostatin areas, one at each end and at different levels of sectioning.

Comparison of serial sections stained with anti-somatostatin and anti-calcitonin showed that the two peptides were present in the same cell (Fig. 2a and b). Somatostatin staining was abolished by prior absorption of the antibody with somatostatin but not by calcitonin. Calcitonin staining was abolished by prior absorption of the antibody with calcitonin but not by somatostatin.

Discussion

In agreement with earlier reports (Hökfelt et al., 1975a; Parsons et al., 1976) we have confirmed that somatostatin immunoreactivity is present in some parafollicular cells of the rat thyroid gland, and that the somatostatin cells are very sparsely distributed.

The use of thin (3 micron) sections stained serially with antibodies to somatostatin and calcitonin has shown that somatostatin, when present, is in the same cells as calcitonin. We cannot determine from this light microscopic study whether or not the two peptides are present in the same granules, but further investigations at the electron immunocytochemical level may clarify this point.

Calcitonin is produced by cells which have been proved conclusively to be derived from the neural crest (Le Douarin and Le Lièvre, 1970; Polak et al., 1974). The finding of somatostatin in the same cell as this peptide, as well as in many other nervous system and gastrointestinal locations, provides further support for the theory that the peptide hormone-producing cells are closely related to each other by a common origin from the neuroectoderm (Pearse, 1969) or, more accurately, from neuroendocrine-programmed epi- (or ecto-) blast. The number of somatostatin cells in each gland is extremely small compared with the number of thyroid follicles and calcitonin cells present. The sphere of influence of somatostatin produced by a single cell would indeed have to be extensive if it were to have a significant local regulatory effect on either calcitonin secretion or on thyroid hormone secretion in the thyroid gland of the adult rat.

There is no structural relationship between calcitonin and somatostatin though the latter shares a common tetrapeptide (Thr-Phe-Thr-Ser) with glucagon and secretin. There is no evidence to suggest that, in the C cells, calcitonin and somatostatin are produced as part of a single prohormone molecule though such a molecule has recently been described in the case of calcitonin (Moya et al., 1975; Van Der Donk et al., 1976). We are thus left as yet with no very acceptable explanation for the presence of the two hormones within the cytoplasm of a single cell type.

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