CALCITONIN AND SOMATOSTATIN ARE LOCALIZED IN DIFFERENT CELLS IN THE CANINE THYROID GLAND

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ABSTRACT

Localization of calcitonin-like and somatostatin-like immunoreactivity in the puppy thyroid gland was examined by the indirect immunoperoxidase procedure. By the use of the double staining method for calcitonin and somatostatin and of alternate immunostaining in serial, adjacent sections, it has been revealed that calcitonin-like and somatostatin-like immunoreactivity occurs in separate cells.

KEY WORDS thyroid gland / parafollicular cells / calcitonin / somatostatin / immunohistochemistry

The localization of calcitonin in parafollicular cells of the mammalian thyroid has been investigated by immunohistochemical techniques (4, 10, 19, 26, 27). Since the immunohistochemical study by Hökfelt and his associates (8), somatostatin has been reported to occur also in the parafollicular cells (23, 28). In the thyroid of the rat (22) and the rabbit (3), it has been reported that calcitonin and somatostatin occur in the same cells. The present paper will report that these polypeptides are localized in separate cells using the puppy thyroid. Since the discovery of the parafollicular cells (1, 21), the thyroid of the dog, especially of young ones, has been known to contain them numerous and is favored material also in recent studies (4, 10-15). Yet the relationship between calcitonin and somatostatin producing cells has not been examined in this species as far as we are aware.

MATERIALS AND METHODS

In the present study, five puppies of both sexes from 1 to about 6 months in age were used. Tissue blocks of the thyroid were fixed in Bouin's fluid for 16-20 hr and embedded in paraffin. Sections, partly cut in continuous series, were treated with the following methods:

1. Serial sections of 2 μ m thickness were

stained alternately by indirect immunoperoxidase procedure using anticalcitonin and antisomatostatin antisera.

2. The double staining method using 4-Cl-1-naphthol and 3,3'-diaminobenzidine-tetrahydrochloride (DAB) (20) was applied to single sections of 4-5 μ m thickness.

Synthetic human calcitonin and synthetic somatostatin (Peptide Institute Protein Research Foundation, Osaka) were used as antigens. Anticalcitonin and antisomatostatin sera were raised in rabbits by injection of either antigen. These primary antisera were kindly supplied by Dr Y. Yamada, Shinrakuen Hospital, Niigata and Dr S. Ito, First Department of Internal Medicine, Niigata University School of Medicine, respectively. Anticalcitonin serum diluted 100 fold and antisomatostatin serum diluted 1,000 fold gave the most favorable reactions. The specificity of the reactions was checked (1) by adding 10 μ g/ml human calcitonin or somatostatin to the primary antiserum beforehand, (2) by using a 100 fold or 1,000 fold diluted normal rabbit serum instead of the primary antiserum, and (3) by the use of only a peroxidase-conjugated secondary antiserum.

RESULTS

The immunoperoxidase method clearly demon-

Fig. 1 Calcitonin-reactive (A) and somatostatin-reactive (B) cells in the puppy thyroid as demonstrated by immunoperoxidase reaction. In micrograph B, a somatostatin-reactive cell extends a cytoplasmic process to interstitial space (arrow). Note that the former cells are by far more numerous than the latter cells.

Fig. 2 Calcitonin-reactive cells (grey) and somatostatin-reactive cells (brown) shown by double staining using 4-Cl-1-naphthol and DAB as substrates

strated in the puppy thyroid that calcitonin-like immunoreactivity and somatostatin-like immunoreactivity were localized in specific cells which all fall into the category of parafollicular cells. However, the cells immunoreactive for calcitonin and somatostatin differed in population and cell shape from each other (Figs. 1-3).

Calcitonin-reactive cells were distributed widely in the interfollicular and parafollicular areas either in groups or singly and were encountered constantly and numerous in every section examined (Fig. 1A). They were usually oval or polygonal in shape.

On the other hand, somatostatin-reactive cells were distributed mostly in the parafollicular position. They were much fewer in number than the calcitonin-reactive cells. Moreover, they varied conspicuously in distribution due to the portion of the thyroid cut: they were numerous in the core part of the organ and very scant in the periphery. Somatostatin-reactive cells were generally polygonal in shape and tended to be much more angular than the calcitonin-reactive cells. Some of them extended slender cytoplasmic processes to the interstitial space (Fig. 1B).

By the use of 4-Cl-1-naphthol for calcitonin and DAB for somatostatin, we could demonstrate that both immunoreactivities, colored grey and brown respectively, were localized in

Fig. 3 Three consecutive sections of the puppy thyroid alternately stained with anticalcitonin (B) and antisomatostatin (A, C) serum. Identical cells on the adjacent sections are indicated by arrows and figures. Occurrence of calcitonin-like and somatostatin-like immunoreactivity in different cells is evident.

different cell types. Alternate use of the substrates could stain the cells reversely. In the serial sections cut 2 μm in thickness we detected calcitonin-like and somatostatin-like immunoreactivity alternately and compared their localization in detail (Fig. 3, A-C). It could thus be confirmed that calcitonin and somatostatin occurred in separate cells. In addition to these cells, we observed parafollicular cells which were not reactive to either antiserum. These were ovoid cells of a considerable number, located mainly in interfollicular positions.

In the present study, we did not count the frequency of the cell types mentioned above but our rough estimation indicated that the cells were decreased in number in the following order: calcitonin-reactive cells, cells not reactive to either antiserum, and somatostatin-reactive cells.

DISCUSSION

The present study showed that somatostatin-reactive cells were separate from calcitonin-reactive cells in the puppy thyroid. As far as we know, two papers are available on the relationship of somatostatin-reactive cells to calcitonin-reactive cells in the thyroid. Noorden *et al.* (1977) demonstrated that in the rat thyroid, somatostatin, when histochemically demonstrable, was in the same cells as those containing calcitonin. In the rabbit thyroid, Buffa *et al.* (1979) demonstrated that at least most, if not all, somatostatin-immunoreactive cells reacted with anticalcitonin antibodies and that approximately 80% of calcitonin-immunoreactive cells reacted with antisomatostatin sera.

Thus, the conclusion of these two papers is that all or a major portion of somatostatin-immunoreactive cells were identical with calcitonin-immunoreactive cells. This conspicuously contradicts the present results in the dog. Whether this discrepancy is simply due to species difference is unknown.

At any rate, the problem on the source cells of the two biologically active messengers, calcitonin and somatostatin, attracts attention with regard to the 'one cell-one hormone' theory which was widely accepted until recently and represented the promotive moment of modern endocrinology. Besides the thyroid, and setting aside the recently revealed occurrence of fragment peptides of a large molecule like β -lipotropin (2), several instances seem to support the possibility that one cell may possess two or more hormones: met-enkephalin and gastrin in the gastrin cell of the human or dog stomach (9,

16, 24), ACTH and gastrin in the gastrin cell of the rat stomach (17, 18), endorphin and glucagon in the A cell of the rat pancreas (7).

It seems worthwhile to mention that DeLellis *et al.* (1978) have distinguished two types of thyroid parafollicular cells containing what they called type I and type II granules respectively, in man on the basis of size and electron density of secretory granules, although this finding obtained in medullary carcinoma and calcitonin cell hyperplasia cannot be correlated directly with the present result in the dog (6). They showed calcitonin-like immunoreactivity in both types of granules by the electron microscopic immunoperoxidase method. Though they do not mention occurrence of somatostatin-like immunoreactivity in the medullary carcinoma they examined, the cells possessing 'the type I granules' are very similar to the normal somatostatin cells of the pancreas as far as we judge from the electron micrograph of the paper. As a matter of fact, there are reports on the occurrence of somatostatin in medullary thyroid carcinoma (5,25). Yamada *et al.* (1977) have reported on the occurrence of somatostatin-like immunoreactive cells in the human, dog, and rat thyroids though they have not precisely examined the topographic relationship of localization between calcitonin and somatostatin (28).

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