Immunohistochemical Demonstration of Calcitonin Gene-Related Peptide and Chromogranin A in C Cells of Sheep Thyroid

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Immunohistochemical studies have demonstrated that C cells of the thyroid gland contain calcitonin and neuron-specific enolase in various animals including the sheep [4, 9–11]. Other polypeptide hormones and proteins, i.e., calcitonin gene-related peptide (CGRP), chromogranin A (CGA), secretory protein-I (SP-I), somatostatin, coexist in the secretory granules of C cells, but immunoreactivity of these hormones and proteins varies from animal species to species [4–8, 11]. In the present study, localization of immunoreactive CGRP and CGA was investigated in the C cells of sheep thyroid.

Five male, 5 female and 5 castrated Suffolk sheep, 2 or 3 years old each, and 2 Suffolk sheep fetuses about 120 days of gestation were obtained from a local slaughterhouse and the facilities for animal supply in Rakuno Gakuen University. Thyroid glands were fixed in 4% buffered paraformaldehyde or Bouin’s solution. Sections were stained with hematoxylin and eosin (HE). Immunohistochemical stainings were performed by the indirect method. Serial sections were reacted with anti-porcine calcitonin (1:1000), anti-rat CGRP (Amersham, England, 1:1500), anti-human CGA (Dakopatts, Denmark, 1:100), or anti-bovine CGA (Immuno Nuclear, U.S.A., 1:1000) sera for 16 hr at 4°C as the first antibody, respectively. As the second antibody peroxidase-conjugated swine anti-rabbit IgG (Dakopatts, Denmark, 1:50) was used for 30 min at room temperature. The preparation and characterization of calcitonin antisera were described previously [9]. For comparison, sections of thyroid glands of cows and rats were also stained for CGRP and bovine CGA. For control, sections were incubated in normal rabbit serum (Dakopatts, Denmark) or phosphate buffered saline, instead of the primary antisera.

It was difficult to distinguish C cells from follicular epithelial cells by HE stain (Fig. 1a). Calcitonin-immunoreactive C cells of the primary adults were distributed throughout the thyroid glands (Fig. 1b). C cells were oval to elongated in shape. They were situated as a single element at the base of follicular epithelial cells or as a group in the parafollicular area (Fig. 1b). C cells were also detected in the parathyroid IV. In the serial sections, calcitonin-immunoreactive C cells were also stained weakly with human CGA and moderately with bovine CGA antisera (Figs. 1d, 1e). Although only a few C cells reacted with CGRP antisera, most of them were negative or weak (Fig. 1c). On the other hand, almost all calcitonin-positive C cells of cows and rats examined in this study were intensely or moderately immunoreactive to both CGRP and bovine CGA. No immunoreaction was observed in all control sections.

In fetuses, almost all C cells were also stained densely with calcitonin antiserum throughout the lateral lobes (Fig. 2a). Most of the C cells reacted moderately with bovine CGA (Fig. 2b) and weakly with human CGA antisera. Only a few C cells were occasionally immunoreactive to CGRP (Fig. 3). Immunoreaction of parathyroid chief cells of adults and fetuses was intense and moderate for bovine and human CGA antisera, respectively.

The present study demonstrated the presence of CGRP and CGA in C cells of the sheep. However, only a few C cells were densely stained with CGRP antisera. The CGRP immunoreactivity of C cells in the sheep was similar to that of hamsters and guinea pigs [5, 11]. On the other hand, it has been shown in many mammalian species including cows, rats, dogs, cats, rabbits, and monkeys that, almost all C cells reveal an intense immunoreactivity for CGRP antisera [5, 6, 10, 11]. However, in the guinea pigs, intense CGRP immunoreaction has not been observed in C cells at any stages of development, indicating that C cells of the guinea pig are lacking in the capacity to synthesize CGRP for life [6]. CGRP arises due to alternative RNA processing events from calcitonin gene [1]. The species difference of CGRP immunoreactivity in C cells indicates that the productive amount of this peptide is different among the animal species examined [5].

Bovine CGA-immunoreactive C cells have been observed in rats, hamsters, mice, and guinea pigs [10, 11]. Bovine SP-I of the parathyroid glands and CGA of the adrenal glands consist of similar proteins, partial amino acid sequences and immunological crossreactivity, and the both two coexist in the secretory granules of the adrenal glands [2]. In the present study, immunoreactivity of bovine CGA in C cells and parathyroid chief cells of the sheep was more intense than that of human CGA. Bovine CGA antisera was also more sensitive in many endocrine cells with secretory granules than monoclonal human CGA antiserum in the various laboratory animals [4]. This results may reflect the difference of amino acid sequence. Moreover, immunoreaction of CGA in C cells of the fetuses was weaker than that of the adults. It has been reported that the bovine CGA immunoreaction is rapidly increased during neonatal periods in C cells of rats, and that an intense immunoreactivity is detected in mature animals [4]. The significance of the difference of CGA immunoreactions between adults and fetuses in the sheep
remains obscure. However it may reflect the difference of the amounts of CGA and/or the method of fixation. Further studies may be required to clarify the ontogeny of immunoreactive CGA in sheep C cells.

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Fig. 2. Two serial sections of a fetal thyroid gland stained immunohistochemically with anti-calcitonin (CT) (a) and anti-bovine CGA (bCGA) (b) sera counterstained with methyl green, respectively. Most of calcitonin-immunoreactive cells are stained with bovine CGA anti-serum. ×120.

Fig. 3. A few C cells of the fetal thyroid gland are stained weakly with anti-CGRP (CGRP) serum counterstained with methyl green (arrows). ×235.