Immunohistochemical Demonstration of Parafollicular (C) Cells in Sheep Thyroid and Parathyroid Glands

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The parafollicular (C) cells synthesize and secrete calcitonin. The distribution of calcitonin was immunohistochemically demonstrated in the thyroid as well as in the internal parathyroid of various mammals, i.e., rabbits [6, 7], cats [6], dogs [7], goats [6, 16], and horses [13, 15]. Neuron-specific enolase (NSE) was also reported to be found in C cells of rats [17, 18, 20, 21], humans [17], dogs [7], guinea pigs [7, 20], and cows [7]. In the present study, the distribution of calcitonin and NSE in the sheep thyroid and parathyroid glands were examined by immunoperoxidase method, as no research had been done in this species.

To obtain the antiserum against calcitonin, porcine calcitonin (total amount 320 MRC/animal, Armour Pharmaceutical Co. Ltd., England) with Freund's complete adjuvant was injected subcutaneously in Japanese white rabbits [9]. The "Western blotting" method was employed to demonstrate the immunospecificity of the obtained antibodies. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli [10]. Then the proteins were transferred to nitrocellulose sheets by the method of Howe and Hershey [2]. A single form of porcine calcitonin immunoreactivity was detected at approximately 3,500 dalton by the "Western blotting" method (Fig. 1).

Fifteen castrated Suffolk sheep, ranging from 1 to 3 years old, were obtained from slaughter houses. Thyroid and external parathyroid glands were fixed in Bouin's solution for 24 to 48 hr, and cut transversely at 3 to 4 mm intervals. Sections were stained with hematoxylin and eosin (HE).

Fig. 1. Characterization of the antibody to porcine calcitonin by immunoblotting. Lanes 1 and 2 show molecular marker proteins. The antigen (porcine calcitonin, lane 3; 0.5 μg, lane 4; 1.0 μg) were submitted to SDS-PAGE and transferred to a nitrocellulose sheet. Lanes 3 and 4 were stained with the rabbit antiserum against porcine calcitonin.

Fig. 2. Calcitonin immunoreactive C cells in the sheep thyroid gland. ×88.
For immunohistochemistry, the indirect immunoperoxidase procedure was used. Serial sections were immunoreacted with the rabbit antiserum against porcine calcitonin at a dilution of 1:1000, and the rabbit antiserum against NSE (Immuno Biological Laboratories, Japan) at a dilution of 1:400 as a primary step.

In the thyroid and internal parathyroid glands, the cytoplasm of C cells, filled with cytoplasmic granules, reacted intensely to anti-porcine calcitonin antiserum (Figs. 2, 3, 6). Almost all C cells were weakly immunopositive to anti-NSE antisera (Fig. 4). In the thyroid glands, C cells, oval to polyhedral in shape, frequently had long cytoplasmic protrusions. They were located mainly in the intrafollicular position and often in
the parafollicular area, but they were never found touching the follicular lumen. Intrafollicular C cells are mostly scattered single elements, and often form chains (Figs. 2, 3). Small groups of 4 to 10 C cells predominantly occurred in the parafollicular area (Fig. 2). C cells were widely distributed in the thyroid glands, but the density of C cells was decreasing toward the periphery of lobes and in the dorsal and ventral poles. No C cells were noticed in the isthmus.

The internal parathyroid gland usually existed within or near the vasculo-stromal hilus, or on the ventro-lateral surface of the thyroid gland, and 12 cases out of 15 contained C cells. C cells usually were distributed in the periphery and were frequently gathered as a large cluster in the central region of the internal parathyroid gland (Figs. 5, 6). The C cells were polyhedral or elongate in shape. No C cells were observed in the external parathyroid glands.

As amino-acid sequence of porcine calcitonin is similar to that of ovine one [14], C cells in the thyroid and internal parathyroid glands of sheep might be strongly positive to anti-porcine antisem. C cells were distributed not only in the thyroid glands but also in the internal parathyroid glands in various mammals, i.e. dogs [5, 6], cats [5, 6], rabbits [5, 6], goats [6, 16], horses [13, 15], and rats [17]. The present study showed that C cells were also present in sheep internal parathyroid glands and widely distributed in the thyroid gland. The distribution of C cells in sheep internal parathyroid glands was similar to that of goats [6, 16], whereas the distribution of C cells in sheep thyroids was different from that of goats [6]. In the goat thyroid, the distribution of C cells was limited to the upper two thirds of the thyroid gland [6].

The number of C cells increased with age after birth in various animals [8, 12, 21]. The incidence of C cell hyperplasia and medullary thyroid carcinoma was high in aged rats [4] and bulls [3, 11]. On the other hand, C cell tumors rarely occurred in young rats [4] and cows [1]. In the present study, the distribution of C cells in the thyroid of adult castrated sheep, ranging 1 to 3 years old, showed no significant difference within the group studied. However, it remained still unclear whether the sex and age were associated with C cell population in sheep thyroid glands.

Concentration of NSE in C cells was somewhat different from species to species [7, 20]. In the present study, most of the C cells immunopositive to anti-porcine calcitonin antiserum were also positively stained with anti-NSE antiserum. Immunoreactivity of NSE in C cells of sheep is similar to that of cows [7]. NSE is mainly present in the cytoplasm of C cells [18]. After chronically induced hypercalcemia, the hyperplastic and hypertrophic C cells of dogs, guinea pigs [7], and rats [19] showed marked decrease in immunoreactive calcitonin, whereas an increase of NSE immunoreactivity was obvious. Thus, the intensity of NSE immunoreactivity in C cells of sheep may be a reflection of the functional activity of the cells in dogs, guinea pigs [7], and rats [19] as reported. Further studies need to clarify the effect of hypercalcemia and the functional correlation between calcitonin and NSE in C cells of sheep thyroid glands.

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REFERENCES

要约

ヒツジの甲状腺および上皮小体におけるC細胞の免疫組織化学的検出（短報）：岡田洋之・松川清・尾谷信春1・横山博1・谷山弘行・湯浅亮1（鶏農学園大学獣医病理学教室、1：獣医学化学教室）——去勢ヒツジ15頭の甲状腺と上皮小体について免疫組織化学的検索を行った。C細胞はウエスタン・プロッティング法により特異性が確認された抗α-カルシトニン抗体に対し強陽性を、市販抗neuron-specific enolase抗体に弱陽性を示した。甲状腺内C細胞は主に中央部に集中しており、峡部には認められなかった。また検索した15例中12例の内上皮小体にC細胞が認められたが、外上皮小体には認められなかった。