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Calcitonin Activity in Ultimobranchial Neoplasms from Bulls

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Abstract. Ultimobranchial neoplasms from 5 bulls that were extracted and assayed individually contained approximately 68% as much calcitonin activity (452 ± 68 MRC mU/g) as thyroid glands from control bulls (663 ± 165 MRC mU/g). These results were consistent with the ultrastructural finding that thyroid neoplasms in old bulls were composed of primitive ultimobranchial cells which contained few mature secretory granules characteristic of parafollicular (C-) cells. Significant difference in calcitonin concentration was not detected between thyroid glands of control cows (617 ± 106 MRC mU/g) and bulls without ultimobranchial neoplasms.

The recent discovery of calcitonin (thyrocalcitonin) (CT) [9, 16] has renewed interest in the endocrine control of calcium metabolism in a number of species. Studies of GODWIN [12] and PEARSE and CARVALHEIRA [27] suggested that the parafollicular or C-cells responsible for secretion of CT were derived from cells, possibly of neurocystic origin, that migrated into the ultimobranchial body during early embryonic development. Further investigations by COPP *et al.* [7, 8] led to the extraction and isolation of CT from ultimobranchial glands of a number of submammalian species.

The ultimobranchial body is derived in higher vertebrates from the terminal branchial pouch in close proximity to other endocrine glands involved in the homeostatic regulation of mineral metabolism. Ultimobranchial tissue varies in postnatal location from within the pericardial transverse septum in fish, near the parathyroid glands in birds, to incorporation within the thyroid tissue in higher mammals. In cattle the ultimobranchial body becomes incorporated in the vasculo-stromal hilus of the thyroid with an ingrowth of cell cords along connective tissue stroma of the gland [19].

Recent investigations have shown that hypercalcemia stimulates hypertrophy, hyperplasia, and increased secretory activity of C-cells in frogs [28], birds [3], rats [22], and cows [2, 32]. GRAY and MUNSON [13, 14] and COOPER and DEFTOS [5] have hypothesized that the physiologic function of CT be to protect against post-prandial hypercalcemia resulting from rapid absorption of intestinal calcium. An elevation in the intake of dietary calcium results in hyperplasia of parafollicular cells in rats [20] and a greater concentrative uptake of amino acids by the ultimobranchial glands in chickens than those on a normal or low intake of calcium [25]. These findings suggest a relationship between intake of dietary calcium and the metabolic activity of C-cells.

Ultimobranchial neoplasms in bulls were originally described by JUBB and MCENTEE [19]. The neoplasms often occurred bilaterally and were derived from cells of the ultimobranchial body. Ultimobranchial tumors were not found in cows of various ages but the neoplasm was of common occurrence (approximately 30%) in old bulls. Recently, KROOK *et al.* [21] suggested an interrelationship may exist between elevated dietary levels of calcium (3.5 to 5.8 times NRC requirements) and the high incidence of ultimobranchial neoplasms in bulls. Reference was made to the exceedingly high incidence of osteopetrosis and spondylosis deformans occurring in older bulls as possible evidence for excess secretion of CT.

Medullary carcinomas of the thyroid gland in man have been shown recently to be associated with increased levels of CT in plasma, urine, and neoplastic tissue [10, 23, 24, 29]. Bone lesions have not been observed in patients with medullary carcinoma despite the excessive CT secretion. Recent ultrastructural investigations indicate this neoplasm is derived from granulated C-cells of ultimobranchial origin in the thyroid [23]. Direct evidence for a similar CT-excess syndrome associated with a thyroid neoplasm has not been described in animals.

The objectives of this investigation were (a) to determine the concentration of CT in ultimobranchial neoplasms from bulls, and (b) to compare these findings with the activity of CT in thyroid glands from normal bulls and cows.

Materials and Methods

Thirteen bulls and 9 cows ranging in age from 5 to 14 years were used in this investigation. The first group of 5 bulls had ultimobranchial neoplasms (3 adenomas, 2 carcinomas) whereas the second group of 8 bulls of similar age had no histopathologic evidence of ultimobranchial or thyroid neoplasia. The third group of 9 adult cows had normal thyroids and were used to compare glandular concentrations of CT between sexes.

Table I. Calcitonin activity in ultimobranchial neoplasms of bulls compared to control bovine thyroid glands, and serum concentration of calcium and phosphorus (± standard error)

Group	Number of animals	Calcitonin MRC mU/g		Serum concentration, mg/100 ml	
				calcium	phosphorus
Bulls with	1	591		9.27	6.69
ultimobranchial	1	265		9.47	6.45
adenoma	1	584		8.98	6.90
		480 ± 108	mean	9.24 ± 0.14	6.68 ± 0.13
Bulls with	1	316		9.89	5.18
ultimobranchial		231 (cervical LnN)			
carcinoma	1	503		8.71	7.31
		409 ± 93	mean	9.30 ± 0.59	6.25 ± 0.75
Control bulls	8	663 ± 165		10.02 ± 0.03	6.75 ± 0.16
Control cows	9	617 ± 106		9.69 ± 0.34	5.02 ± 0.34

Blood and thyroid glands were collected from all animals immediately after euthanasia. Serum was analyzed for calcium by atomic absorption spectrophotometry (PERKIN-ELMER 303) and for phosphorus by the method of FISKE and SUBBAROW [11]. At the time of collection thyroid glands were carefully examined for gross evidence of neoplasia. The neoplasms and normal thyroid tissue were dissected free from each other, frozen in liquid nitrogen (–190 °C), and stored (–20 °C) for subsequent biologic assay. Only the neoplastic tissue was assayed for CT activity in 4 bulls. With 1 bull both the ultimobranchial tumor and adjacent normal thyroid tissue were assayed in parallel for comparative activity (table I). Representative blocks of ultimobranchial neoplasms and thyroid glands were fixed in 10% buffered formalin for histopathologic observation and in osmium tetroxide or glutaraldehyde for ultrastructural evaluation. Thyroid glands from control animals were processed in a similar manner.

The ultimobranchial neoplasms prepared for biologic assay had histopathologic characteristics similar to those described by JUBB and MCENTEE [19] (fig. 1). The neoplastic cells were either separated by strands of fibrous connective tissue into solid nests or arranged into small colloid-containing follicles. The neoplastic cells were cuboidal to polyhedral and had a slightly eosinophilic cytoplasm. Spindle cells occurred in some areas of ultimobranchial carcinomas.

Extracts from the ultimobranchial neoplasms and thyroid glands were prepared individually as described previously [31]. The final supernatant of each extract was lyophilized, sealed under vacuum, and stored at –20 °C. Ampoules of lyophilized extract were reconstituted [26], adjusted to pH 4, and calibrated against a Bovine Thyrocalcitonin Standard (BTS) using a rat biologic assay [4]. The BTS was prepared from thyroid glands of mature dairy cows and calibrated against the Medical Research Council (MRC) Research Standard B for Thyroid Calcitonin in 3 comparative 6-point biologic assays [32]. The activity of the CT standard is such that 10 MRC mU decreases the concentration of serum calcium in rats by 1 mg/100 ml at 65 min after subcutaneous injection. Five-week-old male HOLTZMAN

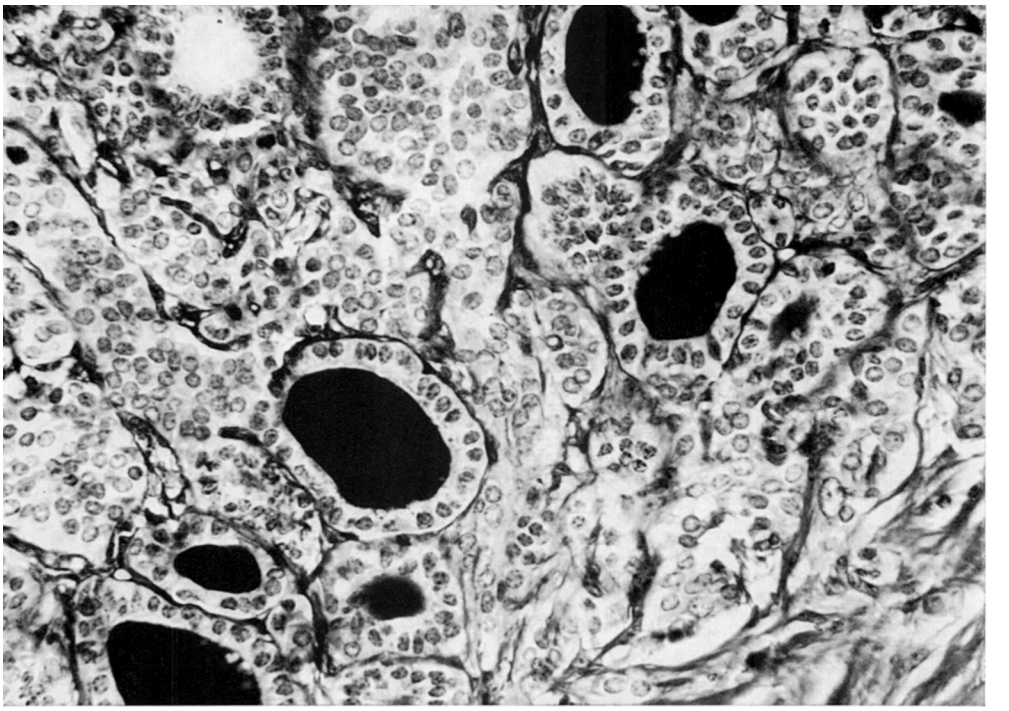

Fig. 1. Ultimobranchial adenoma from the hilus of the thyroid gland from a 12-year-old bull. The neoplastic cells are separated by connective tissue into solid nests or arranged into colloid-containing follicles. Periodic acid-SCHIFF, × 315.

rats[1], weighing 100 to 125 g, were used in the assays. Seven rats were used for each of 2 dose levels of BTS and individual thyroid or tumor extracts. In the 4-point assays the high dose was 4 times the low dose and the maximum subcutaneous dose did not exceed 1 ml. The rats were exsanguinated from the abdominal aorta 65 min after injection and the serum calcium determined by atomic absorption spectrophotometry. The logarithmic dose-response was plotted for each assay and the relative potencies determined [1]. The mean index of precision (λ) was 0.25 for 25 consecutive biologic assays of the BTS.

Results

The serum concentration of calcium and phosphorus in the experimental cattle is summarized in table I. The concentration of serum calcium in bulls with ultimobranchial neoplasms was slightly lower than in either control

[1] Harlan Industries, Inc., Cumberland, Ind.

bulls or cows. Individual assays of extracts revealed the thyroids from 8 control bulls contained 663 ± 165 MRC mU of CT/g. The ultimobranchial neoplasms had a lesser CT concentration than thyroid glands of control bulls. The ultimobranchial adenomas from 3 bulls assayed individually contained 480 ± 108 MRC mU of CT/g whereas the ultimobranchial carcinomas from 2 bulls contained 409 ± 93 MRC mU/g. The difference in CT activity between ultimobranchial neoplasms and thyroid glands from control bulls or cows was not statistically significant.

The bull with the greatest concentration of CT (591 MRC mU/g) in an ultimobranchial adenoma had a serum concentration of calcium and phosphorus of 9.27 and 6.69 mg/100 ml, respectively (table I). The well-differentiated adenomas consisted of 2 nodules (1.0 and 1.2 cm in diameter), one at the hilus of each thyroid lobe. The 2 ultimobranchial adenomas were dissected free, pooled, and extracted separately from the surrounding thyroid tissue. The activity of CT in the adenomas (591 MRC mU/g) was considerably less than in the adjacent non-neoplastic thyroid tissue (1,207 MRC mU/g) assayed in parallel. The remaining 2 adenomas also had lesser CT activity than in thyroid glands of normal bulls (table I).

The lowest serum concentration of calcium (8.71 mg/100 ml) was recorded in a bull with an ultimobranchial carcinoma that contained 503 MRC mU of CT activity per gram (table I). The bull with the most extensive carcinoma had bilateral involvement of both thyroid lobes and widespread metastases to the lung and many cervical, bronchial, and hilar lymph nodes. The primary neoplasm contained 316 MRC mU of CT activity/g and metastases in a cervical lymph node had 231 MRC mU/g.

Control bulls had a mean serum calcium of 10.02 ± 0.03 and phosphorus of 6.75 ± 0.16 (table I). Control cows had slightly lower concentrations of serum calcium (9.69 ± 0.34 mg/100 ml) and phosphorus (5.02 ± 0.34) than the bulls. The thyroid content of CT in control cows (617 ± 106 MRC mU/g) was similar to that of control bulls (663 ± 165 MRC mU/g).

Discussion

This investigation revealed that ultimobranchial neoplasms of bulls contained approximately 68% as great a concentration of CT as thyroid glands from control bulls of the same age. These findings differ from those reported for medullary carcinoma derived from C-cells of the thyroid gland in man. Extracts of medullary carcinomas have been reported to contain from 100 to

5,000 times the CT content compared to normal human thyroid glands [10, 17, 23, 24, 29]. Only one patient showed evidence of hypocalcemia and hypophosphatemia. Levels of CT in thyroid tissue of control cows [32] and bulls were in a similar range as that reported in man [6]. Preliminary studies of plasma CT levels in our laboratory determined by biologic assay after oxycellulose extraction [15] in one bull with an ultimobranchial carcinoma revealed only a slight increase above that of control bulls and normal cows. Plasma levels of hypocalcemic activity are often greatly elevated in patients with medullary carcinomas compared to normal subjects and are further increased by calcium infusions [10, 24, 29, 30].

The results of the biologic assay for CT activity correlated with an electron microscopic evaluation of ultimobranchial neoplasms from 12 bulls. The predominant type of neoplastic cell did not have ultrastructural characteristics of granulated C-cells observed in the thyroid glands of normal bulls and cows. The neoplastic cells frequently assumed a ductular or acinar pattern of arrangement. The cytoplasm contained large mitochondria, aggregates of free ribosomes, and often had an extensive network of concentric microfilaments but few neoplastic cells had membrane-limited secretory granules characteristic of differentiated C-cells [C. CAPEN, unpublished observations]. By comparison, numerous secretory granules have been reported within C-cells composing the CT-secreting medullary carcinoma of man [23]. The neoplastic cells in bulls resembled primitive cells derived from the ultimobranchial duct reported in other species [18].

KROOK *et al.* [21] postulated that the high incidence of ultimobranchial neoplasms and spondylosis deformans in bull was related to the excessive calcium intake (3.5 to 5.9 times NRC requirements). The incidence of thyroid neoplasms and spondylosis deformans was exceedingly rare in cows even though they received a similar large amount of dietary calcium. They suggested the reason for this discrepancy be that cows be better able to utilize the high calcium intake due to calcium requirements of pregnancy and lactation. Although intake of calcium appears to influence the metabolism of C-cells, no relationship has been reported between the levels of dietary calcium and the development of medullary carcinoma in man.

The lower than normal CT activity in ultimobranchial neoplasms of adult bulls detected by biologic assay, lack of ultrastructural characteristics of differentiated C-cells, and relatively frequent occurrence differ from the findings in human patients with C-cell (medullary) carcinoma of the thyroid [10, 23, 24, 29]. The etiologic relationship between thyroid neoplasms and the high incidence of spondylosis deformans and degenerative osteoarthrosis

in older bulls is uncertain. It may be significant that the high plasma levels of CT in human patients with functional medullary carcinoma of the thyroid have not been associated with significant bone lesions. Further investigations of plasma CT levels from bulls with and without ultimobranchial neoplasms will aid in determining the role of the hormone in the pathogenesis of osteoarthropathies.

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