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Calcitonin Deficiency in Primary Hypothyroidism*

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ABSTRACT. The relative lack of sensitivity and specificity of current assays for plasma calcitonin (CT) have made it difficult to study possible CT deficiency conditions. Using a new extraction method that considerably improves the sensitivity of the assay for CT monomer, CT levels were measured before and after a short calcium (Ca) stimulation test (2 mg Ca/kg over 5 min) to determine C-cell secretory reserve in women with autoimmune primary hypothyroidism and normal women. Mean basal plasma CT concentrations were lower in the hypothyroid women [0.9 ± 0.1 (±SEM) pg/ml] than in the normal women (1.5 ± 0.2 pg/ml; $P < 0.01$). Serum Ca increased similarly in both groups, but postinfusion CT levels were lower in the women with primary hypothyroidism (3.8 ± 1.3 pg/ml) than in normal women (15.9 ± 3.0 pg/ml; $P < 0.001$). The functional thyroid status at the time of the study did not influence CT levels; both hypothyroid patients (n = 10) and patients who were euthyroid during T_4 treatment (n = 11) were CT deficient to the same extent. Unlike that in primary hypothyroidism, CT secretion was normal in four patients with hypothyroidism of pituitary origin.

We conclude that the process that causes hypothyroidism in patients with autoimmune thyroid disease can also cause marked CT deficiency. This first demonstration of spontaneous CT deficiency in adults should contribute to the understanding of CT physiology; it also suggests that bone metabolism should be closely monitored during the treatment of primary hypothyroidism (*J Clin Endocrinol Metab* **62:** 700, 1986)

WHEREAS medullary thyroid carcinoma is a well known condition of calcitonin (CT) excess, acquired (not iatrogenic) spontaneous CT deficiency has not yet been reported. Defining such an entity might help in understanding the physiological significance of CT (1), but the relative lack of sensitivity and specificity of available CT assays has so far precluded reliable definition of subnormal levels and thus investigation of CT deficiency.

Body and Heath III (2) recently reported a method for extracting CT from plasma that considerably improved the assay for measurement of CT monomer, the main if not the only form of the hormone active on bone. Using this new technique, we now report that basal CT levels and the CT secretory response to calcium are markedly reduced in patients with spontaneous primary hypothyroidism, making it the first known condition of acquired primary CT deficiency.

Materials and Methods

Subjects

We studied 21 women suffering from spontaneous primary hypothyroidism (defined as elevated serum TSH and low serum thyroid hormone concentrations). To distinguish the consequences of destruction of the thyroid gland from those of hypothyroid status, we divided the patients into 2 groups: group I, 10 patients who were hypothyroid at the time of the study; and group II, 11 patients who were treated with thyroid hormone replacement therapy for at least 4 months and were euthyroid.

For simplicity, all of the following characteristics of the two groups are given in terms of median and range. The ages in groups I and II were, respectively, 68 (58–76) and 60 (35–77) yr. At diagnosis, serum TSH concentrations (normal values, 0.2–5.6 μU/ml) were 26 μU/ml (9.2–>40) in group I and 27 μU/ml (13.7–59) in group II; serum T_4 levels (normal values, 6.4–12.5 μg/dl) were 3.3 μg/dl (0.7–6.9) and 2.9 μg/dl (0.1–6.1), respectively. At the time of the study, serum TSH levels were 11.3 μU/ml (6.9–73.0) in group I and 1.9 μU/ml (0.2–5.0) in group II. Autoimmune destruction of the thyroid gland was the most likely cause of primary hypothyroidism in our patients; a small gland was documented by echography in 17 of 20 cases, 2 patients had a diffuse goiter, and serum antithyroid antibodies were detected in 19 of 21 patients (3). The hemagglutination titers for antithyroglobulin antibodies and antimicrosomal antibodies were, for the whole group, 1:80 (0–1:5120) and 1:6400 (0–1:6.10^6), respectively.

The control group consisted of 24 normal women (mean age, 43 yr; range, 22–65). None was taking any hormone or drug known to affect bone metabolism. All were euthyroid, and none had thyroid enlargement or detectable antithyroid antibodies. As an additional control group, we also studied 4 men (mean age, 62 yr; range 52–63) with hypothyroidism of pituitary origin.

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Hypopituitarism was secondary to an operated pituitary adenoma in 3 and of unknown origin in 1 patient. All 4 patients had documented thyroid, adrenal, gonadal, and somatotropin deficiencies; 3 were receiving thyroid hormone therapy and were euthyroid. They did not have detectable antithyroid antibodies, and the thyroid gland was small (echography) in 3 patients. They were compared to 10 normal men (mean age, 42 yr; range 25–68).

Calcium stimulation test

As previously described (2), we injected 2 mg elemental calcium/kg to recumbent fasting subjects over 5 min. Infusion and sampling were carried out in separate arms. Sampling times were 0 (*i.e.* before the infusion) and 5 min (*i.e.* at the end of the infusion). Informed consent was obtained from every individual.

Analytical methods

CT extraction and RIA. Plasma CT was measured in whole plasma (iCT) and after plasma extraction (exCT). The extraction method was described and validated previously (2, 4). Briefly, up to 20 ml plasma were passed through a disposable silica Sep-Pak cartridge (Waters Associates, Milford, MA). After washing the column, the extracted material was eluted with a mixture of methanol-water (70:30). The eluate was lyophilized in a vacuum centrifuge (Speed Vac, Savant Instruments, Hicksville, NY); the lyophilizates were reconstituted in assay buffer on the day of the assay (2). This extraction method permitted considerable improvement in the sensitivity and specificity of the assay for measurement of CT monomer (2, 4). Recovery of CT monomer is high and consistent, so that correction for loss is not necessary (2, 4). To verify recovery from plasma of patients with primary hypothyroidism, we added purified radioactive CT monomer to normal plasma (n = 12) and plasma from hypothyroid patients (n = 10). The mean recoveries were 92.3 ± 2.4% (±SEM) and 89.8 ± 3.0% (P = NS), respectively.

The CT RIA was that previously described (2, 5), except that we used a new goat antiserum (G-813), generously provided by Dr. Hunter Heath III (Mayo Clinic). That antiserum also recognizes the middle portion of the molecule (determined by using CT fragments donated by Drs. Scheibli and Andreatta, Ciba-Geigy, Basel, Switzerland). The mean intra- and interassay coefficients of variation for appropriate internal reference samples were 8.0% and 16.6%, respectively. All measurements were done in triplicate for both whole plasma and plasma extracts, which, in addition, were assayed at a minimum of two dilutions. Nonspecific binding was measured at each dilution and was less than 6%.

Other measurements. Serum calcium was measured by a colorimetric method using a cresolphthalein complexone (6). The normal range was 9.0–10.2 mg/dl. Serum T_3, T_4, TSH, and antithyroid antibodies were determined by routine laboratory procedures (7).

Statistical methods

The methods used were the two-tailed t test with separate variance estimates, the Kruskal-Wallis nonparametric test, χ^2 analysis, and linear correlations (parametric, expressed by the Pearson coefficient r, and nonparametric where necessary, expressed by the Spearman coefficient r_s) (8).

Results

Serum calcium (Ca)

Basal and postinfusion serum Ca levels were not significantly different among the various groups (Table 1 and Fig. 1, *upper panel*).

Plasma exCT

Basal and postinfusion exCT were higher in the normal women than in the patients with primary hypothyroidism. The differences were significant whether analyzed by t test of mean values (Table 1 and Fig. 1) or by nonparametric testing of the median values [basal exCT, 1.3 *vs.* 0.8 pg/ml ($P < 0.01$); stimulated exCT, 12.5 *vs.* 1.4 pg/ml ($P < 0.001$)]. Only 3 of 21 patients with primary hypothyroidism had an exCT increase of more than 3 pg/ml, compared to 20 of 24 normal women ($P < 0.001$). The same conclusions were obtained when we analyzed the data as the ratio of [stimulated exCT − basal exCT] /[postinfusion Ca − basal Ca] (Δ exCT/Δ Ca) that takes account of individual variations in Ca increase (Table 1 and Fig. 2).

Basal and stimulated exCT were not significantly different in the 10 patients who were still hypothyroid at the time of the study and the 11 patients who were euthyroid during T_4 treatment (Table 1 and Fig. 2). On the other hand, unlike in primary hypothyroidism, there was no CT deficiency in the patients with pituitary hypothyroidism (Table 1).

As previously reported (2), there was no decrease with age in basal or stimulated exCT levels in any group (data not shown). We also confirmed that basal and stimulated exCT were positively correlated (in normal women, r = 0.73 and $P < 0.001$; in primary hypothyroidism, $r_s = 0.52$ and $P < 0.01$). There was a trend to negative correlations between exCT and antithyroid antibody levels (basal exCT *vs.* antiTG, $r_s = -0.47$ and $P = 0.02$; basal exCT *vs.* antimicrosomal, $r_s = -0.34$ and $P = 0.07$; stimulated exCt *vs.* antiTG, $r_s = -0.35$ and $P = 0.06$). Moreover, the eight patients who had no detectable antithyroglobulin had higher basal exCT ($P = 0.02$) and higher stimulated exCT levels ($P = 0.06$).

Plasma iCT

Basal iCT levels were undetectable (<15 pg/ml) in 20 of 24 normal women and in all patients with primary hypothyroidism. Stimulated iCT remained undetectable in 12 of 24 normal women *vs.* 18 of 21 patients ($P < 0.05$).

TABLE 1. CT response to Ca infusion (2 mg/kg over 5 min) in normal women and men, women with primary hypothyroidism, and men with secondary hypothyroidism

	n	Serum calcium (mg/dl)		Plasma exCT (pg/ml)		ΔexCT/ΔCa
		Basal	5 min	Basal	5 min	
Normal women	24	9.3 ± 0.1	10.4 ± 0.1	1.5 ± 0.2	15.9 ± 3.0	14.5 ± 3.4
Women with primary hypothyroidism	21	9.3 ± 0.1	10.5 ± 0.1	0.9 ± 0.1[a]	3.8 ± 1.3[b]	2.2 ± 0.9[c]
Hypothyroid	10	9.4 ± 0.1	10.6 ± 0.1	0.9 ± 0.2	2.4 ± 0.8[b]	1.1 ± 0.4[b]
Euthyroid	11	9.3 ± 0.1	10.5 ± 0.1	1.0 ± 0.1[a]	5.0 ± 2.3[c]	3.2 ± 1.7[c]
Normal men	10	9.3 ± 0.1	10.9 ± 0.3	3.9 ± 0.7	39.1 ± 7.7	28.0 ± 6.7
Men with secondary hypothyroidism	4	9.4 ± 0.1	10.4 ± 0.1	4.3 ± 0.8	51.1 ± 6.5	51.6 ± 5.5[d]

Values are the mean ± SEM. To convert picograms of CT per ml to picomoles per liter, multiply by 0.29253. Δ, change.

[a] $P < 0.05$ *vs.* normal women.

[b] $P < 0.001$ *vs.* normal women.

[c] $P < 0.01$ *vs.* normal women.

[d] $P < 0.05$ *vs.* normal men.

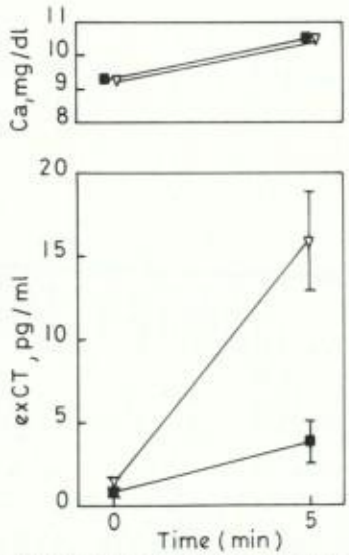

FIG. 1. Calcium stimulation test in 24 normal women and 21 women with primary hypothyroidism. *Upper panel*, Serum Ca before (0 min) and at the end (5 min) of the calcium infusion. *Lower panel*, exCT at 0 and 5 min. Results (untransformed data) are given as the mean ± SEM. ▽—▽, Normal women; ■—■, women with primary hypothyroidism.

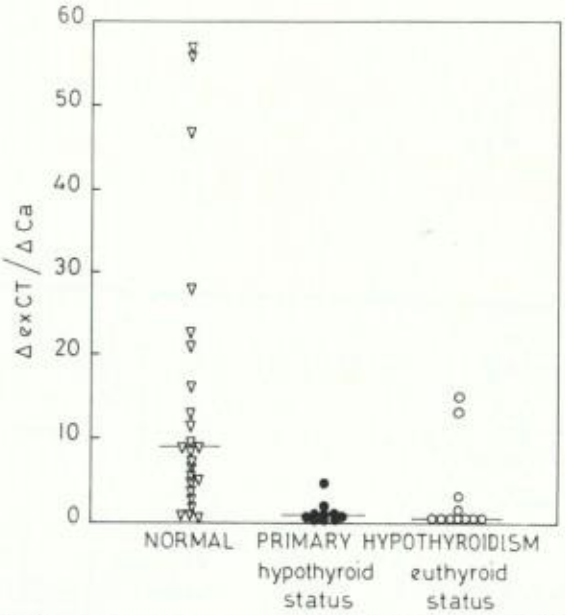

FIG. 2. CT secretory reserve, expressed as the change in exCT/that in Ca (ΔexCT/ΔCa) in normal women (▽) and women with primary hypothyroidism still hypothyroid at the time of the study (●) or euthyroid under thyroid replacement therapy (○). Median values in each group are indicated by *horizontal lines*.

Discussion

We found clear CT deficiency in women with primary hypothyroidism; both their basal plasma concentrations of exCT and their responses to calcium stimulation were markedly reduced. The whole plasma assay also revealed a significantly decreased secretory reserve in the patients with primary hypothyroidism, but only by a difference in the proportion of patients who had detectable iCT responses to calcium infusion. We believe it would be very hazardous to rely only on such findings; an extraction method is mandatory to investigate CT deficiency conditions. Although hypothyroidism reduces bone turnover (9), which slightly elevates circulating PTH and calcitriol (10), our observations cannot be explained on that basis. Indeed, both groups of patients with primary hypothyroidism, whether still hypothyroid or euthyroid during replacement therapy, had similar exCT levels. CT deficiency was thus a consequence of the pathology of the thyroid gland and not of hypothyroidism. This is further evidenced by the absence of CT deficiency in our patients with hypothyroidism of pituitary origin. Moreover, in other situations also characterized by low bone turnover, such as hypoparathyroidism, the CT secretory reserve is elevated (11). Our findings cannot be explained on the basis of age differences between the groups. A

decrease in CT secretory reserve with age has been reported by some investigators (12); however, we previously investigated that question in a large number of individuals by measuring CT before and after plasma extraction, and found no decrement of basal or stimulated CT levels with age in men or women (2, 13). This absence of variation with age was confirmed in the present study.

One can only speculate on the cause of CT deficiency in autoimmune primary hypothyroidism, since we found no data on the pathology of the C-cells in that condition. C-Cells might share antigenic determinants with thyroid follicular cells (14) and be destroyed by a common autoimmune process. This hypothesis is supported by our negative correlations between CT levels and antithyroid antibody concentrations. Similarly, autoantibodies against pancreatic β-cells react with other hormone-producing cells in the islets (15). Since autoimmune endocrine disease may involve several endocrine tissues (16), one could also postulate the existence of distinct autoantibodies against C-cells. On the other hand, the process of lymphocyte infiltration and subsequent fibrosis of the thyroid gland, which are characteristic of autoimmune thyroiditis (17), could lead to nonspecific progressive destruction of the CT-producing cells. Lastly, C-cell function could depend on local trophic factors whose production would be decreased in primary hypothyroidism but not in secondary hypothyroidism.

Primary hypothyroidism is the first reported condition in which spontaneous CT monomer deficiency occurs during adulthood. The existence of this CT deficiency state in adults should help in understanding CT physiopathology. CT deficiency after total thyroidectomy (1, 2) reportedly leads to a decreased bone mass (18). Primary hypothyroidism might similarly predispose to osteopenia; various researchers have indeed reported that correction of hypothyroidism leads to exaggerated osteoclastic activity and reduced bone mass (19–21). In view of the CT deficiency found in the present study, attention should be paid to bone metabolism during treatment of primary hypothyroidism, since pathological bone loss could result from apparently adequate doses of thyroid hormones.

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