Plasma calcitonin response to a calcium clamp in endogenous Cushing’s syndrome

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Abstract. Basal and calcium (Ca) stimulated plasma CT concentrations have been studied in 6 premenopausal females with Cushing’s disease and 3 premenopausal females with Cushing’s syndrome due to an adrenocortical adenoma. Thirteen healthy premenopausal females served as a reference group. A standardized Ca-stimulus was applied to all subjects by means of the ‘calcium clamp’ technique. Plasma immunoreactive CT (iCT) was determined by RIA using a carboxyl terminal antiserum with 8 pg-eq/ml as detection limit.

The patients with Cushing’s disease had normal basal plasma iCT levels and their iCT response during the 2 h calcium clamp was within the reference range in 5 out of 6 patients, the remaining patient showed a slightly exaggerated response. The patients with adrenal adenoma had elevated plasma iCT levels in the basal state but a normal response during the calcium clamp. It is therefore less likely that the bone resorption often seen in Cushing’s syndrome is due to CT deficiency.

In patients with endogenous Cushing’s syndrome osteoporosis is a frequent and sometimes disabling complication which has been attributed to decreased bone formation and increased bone resorption (Riggs et al. 1966; Jowsey & Riggs 1970; Jett et al. 1970; Raisz & Kream 1983, review).

Calcitonin (CT) inhibits the osteoclast activity thereby decreasing the bone resorption (Friedman & Raisz 1965) and it has been proposed that a relative CT deficiency may lead to increased bone resorption and thereby contribute to the development of osteoporosis (Heath & Sizemore 1977; Stevenson et al. 1982; Taggart et al. 1982; McDermott et al. 1983). Since glucocorticoid treatment has been described to suppress plasma CT concentrations (Lo Cascio et al. 1982) we have studied whether the calcium stimulated CT release is suppressed in patients with endogenous Cushing’s syndrome. A sensitive and well characterized radioimmunoassay was used to determine immunoreactive CT (iCT) in unextracted plasma (Bucht et al. 1985). The ‘calcium clamp’ technique previously described was used to achieve a similar Ca-stimulus in all subjects studied (Törring & Sjöberg 1983).

We report the results of a study performed in 6 patients with Cushing’s disease and 3 patients with hypercortisolism due to an adrenocortical adenoma.

Subjects and Methods

Cushing’s syndrome

Nine premenopausal females (32 ± 7 years; mean ± sd, range 21–39 years old) with Cushing’s syndrome participated in the study. The diagnosis was based upon the findings of increased cortisol excretion, absence of normal diurnal variations of plasma cortisol and resistance to suppression with a low dose of dexamethasone (Liddle 1960; Thorén et al. 1975). The origin of the disease was established by means of the high dose dexamethasone (8 mg/day) suppression test (Liddle 1960), plasma ACTH determinations (Thorén 1980) and if necessary (n = 4) the metyrapone test (Liddle et al. 1959). Six of the patients had inappropriate pituitary ACTH secretion (Cushing’s disease) and the remaining patients had an adrenocortical adenoma (Table 1). None of the patients showed evidence of insufficient thyrotrophin or gonadotrophin secretion. X-ray investigation showed normal volume of the pituitary fossae in all patients except in...
Table 1.
Clinical and laboratory data in the patients with Cushing's syndrome.

<table>
<thead>
<tr>
<th>No.</th>
<th>Duration of disease (month)</th>
<th>Urinary cortisol (nmol/24 h)</th>
<th>Plasma ACTH (pmol/l)</th>
<th>Testosterone (nmol/l)</th>
<th>Oestradiol (pmol/l)</th>
<th>Etiology</th>
<th>Treatment</th>
<th>Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>1894 ± 300</td>
<td>106</td>
<td>1.6</td>
<td>121</td>
<td>Cushing's disease</td>
<td>TS</td>
<td>Cure</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>2064 ± 227</td>
<td>23</td>
<td>1.3</td>
<td>102</td>
<td>Cushing's disease</td>
<td>TS + 70Gy</td>
<td>Cure</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>3754 ± 1940</td>
<td>131</td>
<td>1.4</td>
<td>42</td>
<td>Cushing's disease</td>
<td>Adrenalectomy</td>
<td>Cure</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>906 ± 103</td>
<td>—</td>
<td>2.2</td>
<td>114</td>
<td>Cushing's disease</td>
<td>70Gy</td>
<td>Cure</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>1965 ± 415</td>
<td>32</td>
<td>0.3</td>
<td>56</td>
<td>Cushing's disease</td>
<td>TS</td>
<td>Cure</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>559 ± 265</td>
<td>66</td>
<td>1.0</td>
<td>82</td>
<td>Cushing's disease</td>
<td>70Gy</td>
<td>Improved</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>2255 ± 338</td>
<td>&lt;2</td>
<td>0.7</td>
<td>108</td>
<td>Adrenal adenoma</td>
<td>Adrenalectomy left</td>
<td>Cure</td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>470 ± 116</td>
<td>5</td>
<td>1.3</td>
<td>102</td>
<td>Adrenal adenoma</td>
<td>left</td>
<td>Cure</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>1662 ± 56</td>
<td>&lt;2</td>
<td>1.2</td>
<td>58</td>
<td>Adrenal adenoma</td>
<td>left</td>
<td>Cure</td>
</tr>
</tbody>
</table>

Mean ± SD of at least three 24-h periods.
TS: transsphenoidal operation; 70Gy: $^{60}$Co irradiation to the pituitary fossa given with stereotactic technique.

No. 5 in whom it was enlarged. One patient (No. 6) had previously been hemithyroidectomized due to atoxic nodular goitre. Eight patients were investigated by X-ray of the lumbar spines and femoral neck and 5 of them by measurement of the bone mineral content (BMC) of the distal forearm by means of osteodensitometry (Christian- sen & Rodbro 1977). All had signs of osteopenia including decreased Singh Index (Singh et al. 1970) and decreased BMC.

Normal subjects
The reference group consisted of 13 healthy premenopausal females of similar ages (25 ± 5; 19–36 years old). The results of a study in these subjects have been reported previously (Torrying et al. 1985). All had normal BMC of the distal forearms. None of the healthy subjects (or the patients) took oral contraceptives or oestrogens.

Calcium clamp
The 'calcium clamp' technique was applied to achieve a comparable Ca-stimulus in all subjects (Torrying & Sjöberg 1983). The infusion rate of calcium chloride was initially 0.312 mmol calcium$^{++}$/kg body weight/h in all subjects, but was later adjusted at need after frequent B-Ca$^{++}$ determinations. In this way the concentration of ionized Ca in whole blood (B-Ca$^{++}$) could be elevated by approximately 0.25–0.29 mmol/l in the patients with Cushing's syndrome and kept in a steady state around 1.45 mmol/l for the remaining period of 120 min. In this way the Ca-stimulus in the patients with Cushing's syndrome would be identical to that previously obtained in the reference group. All subjects were fasting over-night except for 250 ml of water given just before start and were in a supine position from 45 min before and throughout the infusion period. Samples for determinations of iCT and ACTH in plasma were taken at −30, 0, 15, 30, 60, 90 and 120 min and for testosterone and oestradiol-17β in serum at 0 min.

In addition to the calcium clamp study, the plasma iCT concentrations were determined at basal state 21 to 24 months after adrenalectomy in the 3 patients with adrenal adenomas.

Methods
B-Ca$^{++}$ was measured by means of a Nova 2® (Biomedical Corp, MA 02164. USA; reference range 1.14–1.27 mmol/l, mean ± 2 SD, n = 38) in the healthy females and by means of a ICA 1® (Radiometer %, DK-2400, Den-
mark: reference range 1.14–1.32 mmol/l, n = 44) in the patients with Cushing's syndrome. The fact that different apparatuses were used for the B-Ca++ measurements in the control group has only negligible influence on the results since the Ca-stimulus is based upon measurements performed on the same apparatus in each individual and the performance characteristics are very similar for the two apparatuses. The intra- and inter-assay coefficients of variation (CVa vs CVi) in our hands are 0.01 for both apparatus.

Plasma iCT was determined by means of radioimmunoassay (RIA) (Bucht et al. 1985) using antibodies raised in rabbits by repeated injections of synthetic human calcitonin coupled to bovine serum albumin. The rabbit-antibodies had regional specificity against the carboxyl terminal amino acid sequence of CT. The detection limit was 8 pg-eq/ml and reference range for females ≤ 19 pg-eq/ml (11 ± 4 pg-eq/ml, mean ± 2 sd, n = 40). Serum testosterone was determined after dichloromethane extraction by RIA (Diagnostic Products Corp., CA, USA). The CVa was 0.08 at 2.5 nmol/l and 0.07 at 33 nmol/l, the reference range being 0.5–2.5 nmol/l in females. Serum oestadiol-17β was determined by RIA (EIR, 5305 Würenlingen, Switzerland), the reference range being 50–1420 pmol/l and CVa 0.06 for levels ≤ 1000 pmol/l. Plasma ACTH was measured by RIA (Thorén et al. 1981). The normal range at 8 a.m. was 4–50 pmol/l. Cortisol in 24 h urinary specimens (dU-cortisol) was measured by means of RIA (Farmos Diagn., 20101, Finland), the reference range being 60–300 nmol/24 h.

**Statistics**

Student's t-test for paired data and for means were used if the data were normally distributed and the Mann-Whitney rank sum test if not (Snedecor & Cochran 1980). In the calculations, samples containing undetectable iCT concentrations were assigned the value of the detection limit (8 pg-eq/ml).

**Results**

Patients with Cushing's disease: the basal B-Ca++, the increase in B-Ca++ (ΔB-Ca++) and the steady state levels were identical in patients with Cushing's disease and healthy subjects (Fig. 1).

The individual iCT curves (Fig. 2) for 5 of the patients with Cushing's disease were all within the reference range but numerically below the mean value curve of the control group, and the hemithyroidectomized patient (No.6) had the lowest iCT values. The remaining patient (No. 2) showed a slightly exaggerated response.

The mean basal plasma iCT level (iCT₀) was

![Graph](image_url)

**Fig. 1.**

The concentration of ionized calcium in whole blood (B-Ca++, mean ± sd) during a 120 min calcium clamp in 6 females with Cushing's disease (●●●●●) and in 13 healthy females of similar ages (○○○○○).
The plasma calcitonin (iCT) response to a 120 min calcium clamp in patients with Cushing’s disease (—, n = 6), and Cushing’s syndrome due to an adrenal adenoma (-----, n = 3). The dotted lines indicate the reference range (mean ± SD) of the iCT response observed in 13 healthy young premenopausal females. Samples containing undetectable concentrations of iCT (<8 pg-eq/ml) are depicted within the shaded area.

normal in patients with Cushing’s disease. Neither the mean increase in plasma iCT after the first 15 (Δ₁₅ iCT) and 30 min (Δ₃₀ iCT) of the infusion nor the maximal increase (Δₘₐₓ iCT) differed from that of the reference group when patient No. 6 was excluded (Mann-Whitney rank sum test).

No correlation was found between serum testosterone or oestradiol-17β and iCT. Δ₁₅ iCT, Δ₃₀ iCT and Δₘₐₓ iCT in patients with Cushing’s disease and in the control group. Nor did the serum testosterone level (1.3 ± 0.6 nmol/l; mean ± SD) differ significantly from that of the control group (1.9 ± 0.7, n = 10). The serum oestradiol-17β concentration (86 ± 32 pmol/l) was lower than in the control group (367 ± 233, n = 10, P < 0.01, Mann-Whitney rank sum test). The individual testosterone and oestradiol-17β-values are shown in Table 1.

The patients with an adrenal adenoma had normal but slightly higher basal B-Ca++ levels, 1.23–1.29 (mean = 1.26) mmol/l than the patients with Cushing’s disease. The ΔB-Ca++, 0.21–0.30 (0.25) mmol/l was similar to the ΔB-Ca++ in the two other groups as the steady state levels were held correspondingly higher, 1.50–1.51 (1.51) mmol/l.

Plasma iCT concentrations in patients with an adrenal adenoma were elevated at basal state and during the ‘calcium clamp’ (Fig. 2). Their iCT increments were similar to those of the references group. The basal serum testosterone and oestradiol-17β concentrations were within the same range as in the patients with Cushing’s disease (Table 1).

At the following up after adrenalectomy, the basal plasma iCT concentrations had been normalized in No. 8 (17 pg-eq/ml) and reduced to 50–70% in patients No. 7 and 9 (31 and 72 pg-eq/ml, respectively).

No correlation was found between mean dU-cortisol and Δ₁₅ iCT, Δ₃₀ iCT or Δₘₐₓ iCT in the Cushing’s disease or the group comprising all patients with Cushing’s syndrome. Plasma ACTH showed no consistent changes during the calcium clamp in patients with Cushing’s syndrome.
Discussion

In patients with Cushing's disease the basal plasma iCT concentrations were within the reference range. This finding is consistent with a previous report by Lamberts et al. (1980). The Ca-stimulated iCT response was also within the reference range in all of patients with Cushing's disease except in one who had an exaggerated response. This indicates that the CT secretion mediated by changes in the blood Ca level is normal in these patients. It is therefore not probable that a relative CT deficiency plays a major role for the development of osteoporosis in Cushing's disease.

In contrast to our finding in Cushing's disease, decreased basal CT levels have been reported by Lo Cascio et al. (1982) in patients treated with glucocorticoids. The cause for this discrepancy is unclear but androgens may play a role. We have recently observed a positive correlation between basal serum testosterone levels and plasma iCT concentrations in males (Törnring et al., in press) and it is well known that glucocorticoid treatment decreases the adrenal androgen production due to its suppressive effect upon the ACTH secretion.

Elevated plasma iCT concentrations were found in 3 patients with adrenocortical adenoma. The Ca-stimulated plasma iCT responses in these patients, however, were numerically similar to those of the control group and those of the patients with Cushing's disease. After extirpation of the adenoma, the plasma iCT concentrations decreased but still remained elevated in 2 of the 3 patients. It is not excluded that one of the adenomas secreted CT or any factor(s) with stimulatory properties upon the CT secretion. CT secretion from ectopic ACTH producing tumours causing Cushing's syndrome has been reported (Himsworth et al. 1977; Asa et al. 1980) and immunohistochemical studies may give some indications whether the adenoma in our patients contained CT producing cells. The question arises whether the not normalized basal plasma iCT levels might be due to thyroid derived CT. We find it unlikely that these patients have medullary thyroid cancer as their iCT responses to calcium were not exaggerated despite elevated basal iCT levels. However, only careful follow-up in the future will make it possible to exclude medullary thyroid cancer or C-cells hyperplasia in these patients.

In conclusion, patients with Cushing's disease had basal and Ca-stimulated plasma iCT concentrations similar to those of the reference group. These findings indicate that the osteopenia frequently seen in patients with Cushing's disease most likely is caused by other factors than a relative CT deficiency. The observation of increased plasma iCT levels during basal conditions in the 3 patients with a cortisol producing adren al adenoma suggests the use of elevated plasma iCT levels in Cushing's syndrome as a marker of adrenocortical adenomas. This would need further confirmation in larger materials including patients with hypercortisolism due to adrenal malignancy and ectopic CRF or ACTH secretion.

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References


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