Original Paper

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Suppression by Calcium of Serum Levels of Intact Parathyroid Hormone in Patients with Primary Hyperparathyroidism

Key Words
Hyperparathyroidism
Parathyroid hormone
Calcium

Abstract
Primary hyperparathyroidism (pHPT) is associated with a right-shifted relation between parathyroid hormone (PTH) secretion and calcium. However, it is also possible that a decreased suppressibility of PTH secretion by calcium is important for maintaining hypercalcemia in pHPT. We therefore compared the suppression of serum levels of intact PTH induced by a 1.5-gram oral calcium load in patients with mild pHPT with that in healthy subjects. The calcemic response to the oral calcium load was the same in the two groups and did not correlate with the degree of PTH suppression or to serum levels of vitamin D metabolites. It was found that serum levels of intact PTH were less suppressed by the oral calcium load in patients than in healthy subjects ($p < 0.01$), but with a considerable overlap between the two groups. The suppression of serum levels of intact PTH was correlated both to baseline serum total calcium levels ($r = -0.55$; $p < 0.05$) and osteocalcin levels ($r = -0.69$; $p < 0.05$) in the patients, but no such correlations were seen in the controls. We conclude that patients with pHPT have a decreased suppressibility of PTH secretion by calcium. Although this reduced suppressibility could be important for maintaining hypercalcemia in some patients with pHPT, it does not aid in the differential diagnosis between patients with mild pHPT and healthy subjects.

Introduction

The serum level of parathyroid hormone (PTH) is dependent on the serum calcium level in a steep inverse sigmoidal fashion [1–3]. The relation between the two parameters is mathematically described by four parameters, the maximum PTH level, the minimum PTH level, the set point (the calcium concentration causing half-maximal inhibition of PTH secretion), and parameter B, a parameter related to the slope of the curve at the set point [4] (fig. 1).

In primary hyperparathyroidism (pHPT), the inverse sigmoidal relationship between PTH and calcium is retained, although the set point is shifted to the right [5–7]. This increased set point has been suggested to play a key role in maintaining hypercalcemia in pHPT [4, 7]. However, there are also data, both from studies in vitro [5, 6, 8, 9] and in vivo [10, 11], demonstrating that a decreased suppressibility of PTH secretion by calcium could be of some importance for the hypercalcemia seen in pHPT. Whether patients with mild pHPT also exhibit impaired calcium-induced suppression of PTH secretion and, if so,

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Table 1. Clinical and biochemical data for serum factors on the patients with phPT and the healthy control subjects

	Patients (n = 14)	Healthy subjects (n = 10)	Normal range	p
Calcium, mmol/l	2.62 ± 0.13	2.33 ± 0.09	2.20–2.60	<0.001
Ionized calcium, mmol/l	1.47 ± 0.06	1.25 ± 0.04	1.17–1.29	<0.001
Intact PTH, pmol/l	7.8 ± 3.1	3.2 ± 1.4	1.0–5.0	<0.001
$25(OH)D_3$, nmol/l	60 ± 18	60 ± 17	20–100	NS
$1,25(OH)_2D_3$, pmol/l	89 ± 39	81 ± 16	36–120	NS
Osteocalcin, µg/l	4.5 ± 1.9	2.2 ± 1.4	1.8–6.6	<0.01

Results are given as the mean ± SD. p = The probability level of random difference between the groups; NS = not significant.

whether this might be an aid in the differential diagnosis against normality, has not been established. A previous investigation has suggested this to be the case [16]. However, that study used an assay for the amino terminal PTH, and thus did not solely measure the intact hormone. In this study we have therefore examined the suppression of serum PTH levels by an oral calcium load in patients with mild pHPT and in healthy subjects, using a highly sensitive assay for intact PTH. The study also examined the association between the suppressibility of PTH by calcium and biochemical markers of PTH activity in pHPT, i.e., the extracellular calcium concentration and serum levels of osteocalcin [12, 13] and dihydroxycholecalciferol [14].

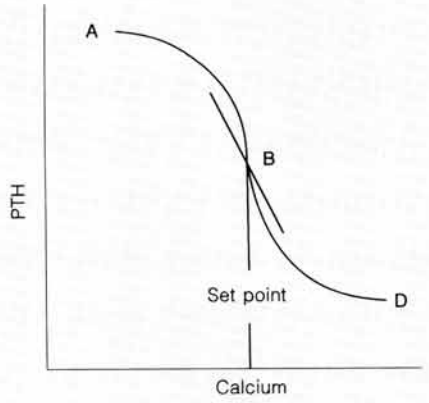

Fig. 1. The sigmoid curve generated by the four-parameter equation. Intact PTH is shown as percent of maximal secretion. A = Maximum PTH release during hypocalcemic stimulation; B = a parameter related to the slope of the mathematical function at the set point; D = minimum secretory rate during hypercalcemic inhibition.

Material and Methods

Patients and Subjects

The study was approved by the Ethical Committee of the Faculty of Medicine at the University of Lund. Informed consent was obtained in all cases. Fourteen patients with a mean age of 63 ± 14 (SD) years and with suspected pHPT and ten healthy control subjects without medication and with a mean age of 48 ± 16 years entered the study. Biochemical data on the two groups are shown in table 1. All patients had mild hypercalcemia (<2.90 mmol/l). Six of the patients had serum levels of total calcium within the normal range and two of the patients had normal serum levels of intact PTH (table 2).

All fourteen patients underwent neck exploration. A single parathyroid adenoma with a mean weight of 0.64 ± 0.37 g was found in all 14 patients.

Oral Calcium Load Test

After an overnight fast, one indwelling catheter for blood sampling was inserted into an antecubital vein. After two baseline samples for determination of the serum levels of ionized calcium and of intact PTH had been taken, 1.5 g calcium (Calcium-Sandoz® effervescent tablet 0.5 g; Sandoz, Basel, Switzerland) was administered p.o. dissolved in 100 ml of water. For the next 2 h, blood samples for determination of serum levels of intact PTH and ionized calcium were obtained at half-hour intervals. The samples were immediately placed on ice, and centrifuged within 45 min. Serum was separated and frozen at −20 °C before analysis.

Analysis

Serum levels of intact PTH were measured with the N-tact PTH IRMA assay (Incstar, Stillwater, Minn., USA). The sensitivity of this assay is 0.13 pmol/l, the between-assay variation is <11% and the within-assay variation is <6%. The reference range is 1.0–5.0 pmol/l.

Table 2. Data on the 14 patients with primary hyperparathyroidism

Patient No.	Serum calcium mmol/l	Serum ionized calcium mmol/l	Serum PTH pmol/l	PTH suppressibility[1] %
1	2.60	1.45	6.2	58
2	2.47	1.42	7.5	43
3	2.63	1.45	10.2	34
4	2.70	1.52	9.8	13
5	2.48	1.41	5.7	11
6	2.84	1.56	6.2	8
7	2.59	1.44	12.1	31
8	2.56	1.50	15.0	55
9	2.66	1.57	8.3	19
10	2.76	1.46	5.6	38
11	2.50	1.43	7.0	44
12	2.74	1.44	3.6	−17
13	2.69	1.54	4.4	20
14	2.40	1.38	7.0	43

[1] The suppressibility of PTH secretion by the oral calcium laod in the ten healthy control subjects was 43 ± 32%.

Serum concentrations of osteocalcin (bone gla protein) were measured with the commercially available Incstar Osteocalcin^{125} RIA kit. The sensitivity of this assay is 0.2 µg/l, and the reference range is 1.8–6.6 µg/l.

Serum levels of 25-hydroxycholecalciferol [25(OH)D_3] were measured by high-pressure liquid chromatography. The reference range is 20–100 nmol/l.

Serum levels of 1,25-dihydroxycholecalciferol [1,25$(OH)_2D_3$] were measured with a radioreceptor assay (Incstar), the reference range of which is 36–120 pmol/l.

Serum ionized calcium (Ca_i) concentrations were analyzed with an ion-selective electrode (Radiometer, Copenhagen, Denmark).

Serum concentrations of calcium and albumin were assayed in a routine autoanalyzer.

Calcium concentration was corrected for serum albumin according to the formula Ca_c (mmol/l) = measured calcium (mmol/l + 0.02*[40 minus measured serum albumin concentration (g/l)].

Statistics

The results are expressed as means ± SD. The Mann-Whitney U test, Wilcoxon signed-rank test, two-way analysis of variance (ANOVA), and Spearman rank correlation coefficient were used for statistical evaluation. A probability level of random difference of $p < 0.05$ was considered significant.

Results

During the oral calcium load, serum levels of ionized calcium increased from 1.47 ± 0.06 mmol/l to 1.56 ± 0.06 mmol/l at 120 min or by 0.09 ± 0.04 mmol/l in patients ($p < 0.01$), and from 1.25 ± 0.04 mmol/l to 1.32 ± 0.05 mmol/l or by 0.07 ± 0.03 mmol/l in healthy subjects ($p < 0.05$; fig. 2). Analysis of variance did not reveal any difference in the calcemic response between the two groups. The maximal calcemic value during the test did not correlate to the degree of suppression of the serum levels of PTH by calcium in either patients or controls nor to the serum levels of vitamin D metabolites.

Serum levels of PTH decreased from 7.8 ± 3.1 pmol/l to 5.3 ± 1.9 pmol/l at 120 min or by 29 ± 21% in patients ($p < 0.01$; table 2) and from 3.2 ± 1.4 pmol/l to 1.6 ± 1.0 pmol/l or by 43 ± 32% ($p < 0.01$) in healthy subjects (fig. 3). Analysis of variance demonstrated that patients had a decreased suppressibility of PTH compared to healthy subjects ($p < 0.01$). Suppression of serum levels of PTH at 120 min in the patients showed an inverse correlation to baseline serum levels of total calcium ($r = -0.55$; $p < 0.05$; fig. 4) and osteocalcin ($r = -0.69$; $p < 0.05$; fig. 5).

Discussion

The present investigation demonstrates a decreased suppressibility of serum levels of PTH by an oral calcium load in patients with mild pHPT compared to healthy subjects when expressed as a percentage. The reduced suppressibility is not evident when expressed as absolute changes since the patients have a higher parathyroid cell

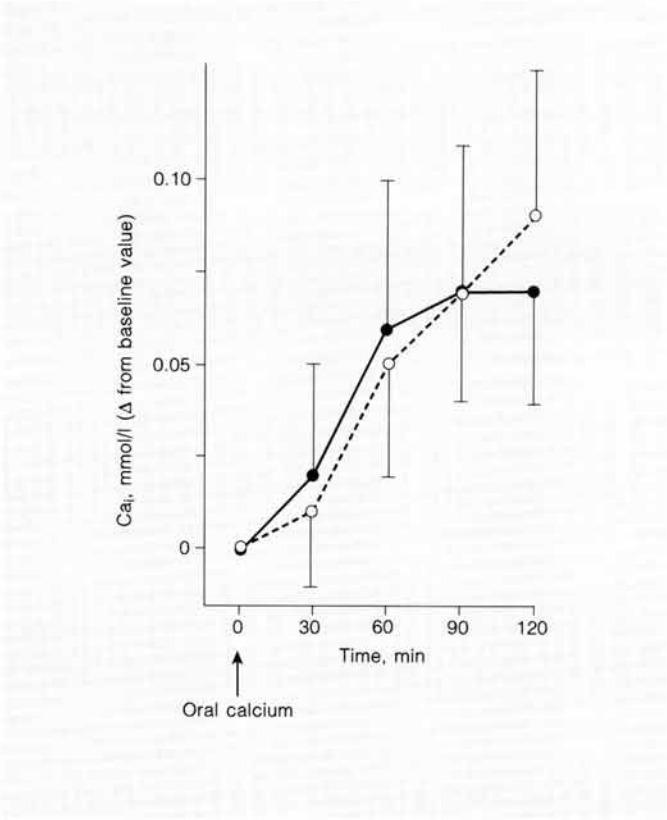

Fig. 2. Changes in serum levels of ionized calcium during the oral calcium load test in patients (○) and healthy subjects (●). Means ± SD are shown.

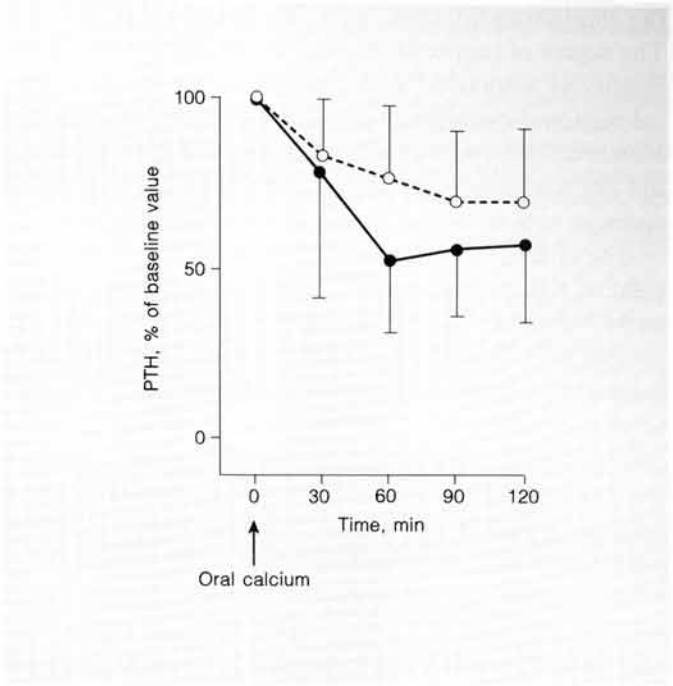

Fig. 3. Changes in serum levels of PTH during the oral calcium load test in patients (○) and healthy subjects (●). Means ± SD are shown.

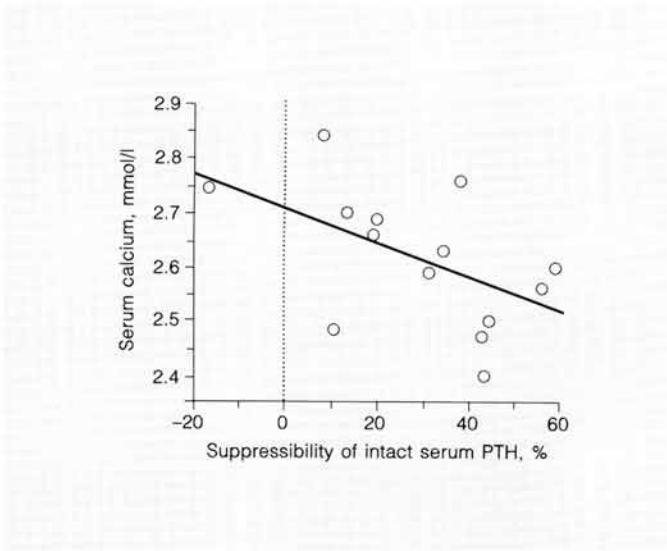

Fig. 4. Correlation between the suppression of serum levels of intact PTH induced by the oral calcium load test at 120 min and baseline levels of serum calcium in the 14 patients with pHPT ($r = -0.55$; $p < 0.05$).

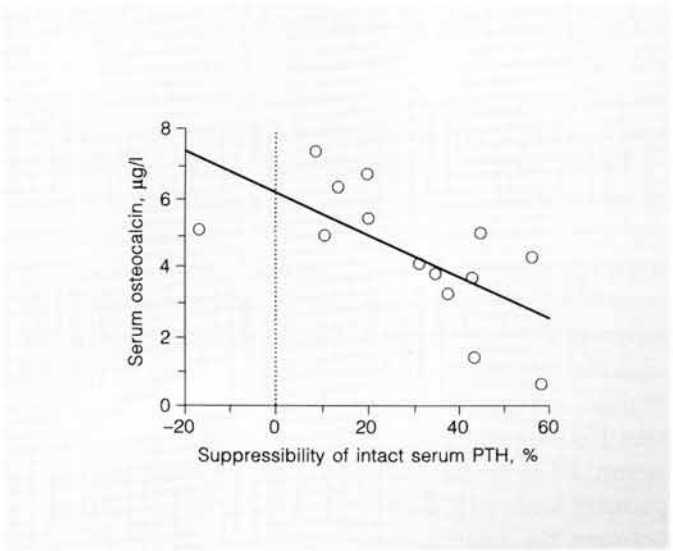

Fig. 5. Correlation between the suppression of serum levels of intact PTH induced by the oral calcium load test at 120 min and baseline levels of serum osteocalcin in the 14 patients with pHPT ($r = -0.69$; $p < 0.05$).

mass. Thus, our study shows that pHPT is associated with impaired inhibition of PTH secretion by calcium. This corroborates the results of earlier investigations, estimating PTH or PTH activity with less sensitive methods than the immunoradiometric assay for intact PTH [15, 16]. The degree of suppression of the serum levels of PTH was negatively associated with baseline serum levels of total calcium and osteocalcin but did not correlate with age. However, the maximal calcemic response during the test did not correlate with the suppressibility of PTH by calcium, or with serum levels of vitamin D metabolites.

The characteristic change of the set point (shift to the right of the inverse sigmoidal relation between calcium and PTH) which is seen in patients with pHPT, has previously been demonstrated to be of great importance for maintaining hypercalcemia in these patients [4]. The high set point has moreover been shown to correlate with baseline values of calcium both in vitro [7] and in vivo [11]. Investigations in vitro have, however, also disclosed a decreased suppressibility of PTH secretion by calcium in some abnormal parathyroid glands [5, 6, 8]. Further, an in vivo investigation has suggested that the change in the slope of the curve of PTH secretion might be dissociated from alterations in its position [11]. Thus, at least for some patients, besides a set point error, the degree of suppressibility could be important in maintaining hypercalcemia in pHPT. Our study supports this hypothesis, since the suppression of PTH by the oral calcium load correlated with baseline serum levels of total calcium. However, the oral calcium load did not evoke a larger increase in serum levels of ionized calcium in patients compared to the increase seen in the control subjects. Hence, the decreased suppressibility seems not to be of *major* importance for the hypercalcemia in most patients with pHPT.

There was considerable overlap in the suppressibility of serum levels of PTH between the patients with pHPT and the healthy subjects. Therefore, in agreement with some investigators [17] but not all [15], the decreased suppressibility did not add any further information in the differential diagnosis against normal subjects beyond what could be deduced from baseline serum levels of total and ionized calcium and intact PTH. One possible explanation for this could be, as with the relative increase in serum PTH levels during the EDTA infusion test in patients with pHPT [10], that there is no association between the relative suppressibility and weight of the excised parathyroid adenoma on the one hand and baseline serum levels of intact PTH on the other.

It has previously been suggested that serum levels of osteocalcin reflect the status of metabolic bone turnover, particularly bone formation, in parathyroid disease [13]. Furthermore, patients with pHPT are known to have moderately decreased bone density, and the bone density is negatively associated with serum levels of osteocalcin [18]. We found that the suppression of serum levels of PTH correlated to baseline serum osteocalcin levels. The significance of this correlation remains to be established.

Conceptually, a decreased suppressibility of PTH secretion by calcium could depend on an increase in the minimal secretion of PTH, and/or a decrease in the slope of the curve above the set point (fig. 1). Since we have previously demonstrated that PTH secretion can be suppressed almost down to zero in vivo [19], and it has been demonstrated in vitro that PTH secretion from most parathyroid adenomas can be suppressed by more than 50% [6], it seems likely that the decreased suppressibility demonstrated by the oral calcium load primarily reflects a relative insensitivity of PTH secretion for calcium concentrations above the set point. Thus, the reduced suppressibility indicates a flatter slope of the curve, i.e., a decrease in parameter B.

In summary, we have demonstrated that patients with mild pHPT have a decreased suppressibility of PTH by oral calcium, which could be of importance for maintaining hypercalcemia and for bone involvement in some patients. However, the reduced suppressibility does not aid in the clinical diagnosis in patients with mild pHPT.

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