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# Direct *in vivo* assessment of parathyroid hormone-calcium relationship curve in renal patients

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**Direct *in vivo* assessment of parathyroid hormone-calcium relation curve in renal patients.** Secondary hyperparathyroidism (SHP) is a well documented finding even in the early stages of chronic renal failure (CRF). A sigmoidal relationship, fitting a four parameter model, links PTH secretion rate and calcium concentration changes. To our knowledge, PTH secretory parameters have only been studied in uremic patients who are in dialysis treatment. As a result of these studies, a possible role for derangement in setpoint values (that is, the serum calcium concentration corresponding to the mid-range value on the sigmoidal curve) has been suggested in the pathogenesis of SHP in CRF. Our study was undertaken to gain insight into the calcium-PTH relationship curve in the first course of CRF and to assess whether a change in any of the secretory parameters is related to the beginning of SHP. We studied 27 male renal patients with a variable degree of renal function (creatinine clearance 12 to 164 ml/min) and 9 control subjects. In all patients and controls the following parameters were evaluated: (1) basal $1,25(OH)_2$ vitamin D, 25(OH)vitamin D, calcitonin (CT), intact PTH; (2) GFR by $Cr^{51}$EDTA clearance; (3) the sigmoidal PTH-ionized calcium relation curve, by means of a hypocalcemic stimulating test ($Na_2$-EDTA 37 mg/kg body weight/2 hr) and a hypercalcemic test (Ca gluconate giving 8 mg/kg of body weight/2 hr of Ca element), performed on two consecutive days. The main results were: (1) the progressive reduction of GFR was accompanied by an increase in the maximum secretory capacity of PTH, without any change in setpoint values; (2) in addition to the already known factors, CT seems to be, in some as yet undefined way, related to PTH hypersecretion in the course of CRF.

Increased parathyroid hormone (PTH) secretion develops early in the course of chronic renal failure (CRF) [1, 2], owing to multiple pathogenetic factors, with a major role played by phosphate retention, reduced availability of calcitriol and/or of its parathyroid gland receptors, and skeletal resistance to the calcemic action of PTH [3–18].

It is also known, by *in vivo* and *in vitro* studies, that PTH secretion varies as a function of serum calcium concentration in a curvilinear fashion that resembles a sigmoidal curve [19–22], characterized by four main parameters: maximal PTH ($PTH_{max}$) which represents the maximum stimulated PTH levels; minimal PTH ($PTH_{min}$) representing maximum PTH inhibition; the slope of the curve in the straight part, which provides information on the sensitivity of the parathyroid glands; and the setpoint, that is the calcium concentration corresponding to half-maximal inhibition of PTH (that is, the midrange value).

Brown et al [20, 21] demonstrated in *in vitro* studies that the calcium-PTH relation curve was shifted to the right in parathyroid glands obtained from patients with both primary and secondary hyperparathyroidism. Several authors have also examined PTH secretion kinetics in patients with CRF [23–27]. Felsenfeld et al [26] reported different patterns of calcium-mediated PTH secretion among patients with different types of renal osteodystrophy. Delmez et al [24] and Dunlay et al [25] demonstrated that vitamin D administration in dialyzed patients induced a marked suppression of PTH levels, together with a shift of the sigmoidal calcium-PTH curve to the left and downward. From these studies, it could be supposed that changes in the PTH secretory parameters, dependent or not on vitamin D deficiency, might play a causative role in secondary hyperparathyroidism (SHP) in CRF. On the other hand, more recently, Ramirez et al [27] were not able to find any change in the setpoint values of uremic patients.

However, all the above studies were performed with uremic patients in dialysis treatment. Furthermore, the methodology for the assessment of the secretory parameters was quite different in the various studies, preventing the possibility of a direct comparison of the results.

To our knowledge, no information is yet available on PTH secretion kinetics during the development of renal failure.

The current study was undertaken to investigate calcium-mediated PTH secretion in the early course of CRF and, secondly, to assess whether the beginning of SHP is in some way related to changes in any of the PTH secretory parameters.

## Methods

### *Patients*

The study was performed on 27 male patients, aged 20 to 72 years, who were suffering from biopsy-proven glomerulonephritis (16 patients), renal vascular disease with hypertension (4 patients), chronic interstitial nephritis (7 patients), Alport's disease (1 patient), and 9 normal male subjects, aged 28 to 54 years.

The patients' renal function was substantially stable in the six months prior to the study, ranging from 12 to 164 ml/min of creatinine clearance; their urinary protein excretion was under 3.5 g/day.

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The four hypertensive patients had clonidine as an anti-hypertensive drug. None of them received vitamin D compounds, oral phosphate binders or diuretics for at least one month preceding the study. The protein and phosphate content of the diet was not restricted in patients with creatinine clearance above 35 ml/min, but was reduced to 0.6/kg body weight of proteins and 800 mg of phosphate, plus 1000 mg of supplemented calcium, in the other patients.

The study was carried out from October to March, the least sunny months in our country. Before performing the study, informed consent was obtained from each patient.

The study consisted of a basal evaluation of serum chemistry, creatinine clearance, and 24 hr electrolytes (at least 2 consecutive samples). On the last day of basal evaluation, the following parameters were also evaluated: intact PTH, $1,25(OH)_2$ vitamin D, 25(OH) vitamin D, calcitonin (CT).

The following day (first study day), GFR was measured by $Cr^{51}$-EDTA clearance in each patient. Briefly, 1 MBq $C^{51}$-EDTA (Sorin Biomedica, Saluggia, Italy) was injected i.v. in a peripheral vein of the arm in one minute; thereafter blood samples were collected at 120, 180, 240 and 360 minutes from the peripheral vein of the controlateral arm. Calculation of GFR was performed according to Sapirstein et al [28].

On the second day, a hypocalcemic test was performed by $Na_2$-EDTA i.v. infusion. Briefly, $Na_2$-EDTA was added to 5% dextrose solution, in such an amount that about 37 mg/kg of body wt were infused in 120 minutes, at a constant rate of 4.2 ml/min of solution, through an indwelling needle. Twenty milliliters of 1% lidocaine were added in the flask to avoid local pain. Blood samples were taken from the controlateral arm at times 0, 5, 10, 15, 30, 45, 75, 105, and 120 minutes to evaluate ionized calcium and intact PTH; CT was also measured in the 0 and 120 minute blood sample.

On the third study day, an infusion test with calcium gluconate (calcium element 8 mg/kg of body wt in 2 hr) was performed; a blood sample was obtained at times 0, 15, 30, 60, 90, and 120 minutes to evaluate ionized calcium and intact PTH. The basal calcium concentrations on the day of calcium infusion substantially overlapped the ones preceding the hypocalcemic test ($1.26 \pm 0.05$ vs. $1.25 \pm 0.05$, $P$ = NS).

Vital signs were monitored every 15 minutes during and for one hour after both infusion tests.

Calculations of the PTH secretory parameters were performed as follows. The maximal stimulated PTH ($PTH_{max}$) was the maximal reached PTH value during $Na_2$-EDTA infusion; this value was reached in all patients between 45 and 75 minutes, and then PTH values remained stable or decreased. The maximal inhibited PTH ($PTH_{min}$) was the lowest PTH obtained during i.v. Ca infusion test; this value was reached between 30 and 60 minutes, and thereafter PTH levels remained substantially unchanged. Sensitivity (Sens) was calculated from the slope of the linear part of the sigmoidal curve described by the relationship between the percentage of $PTH_{max}$ values of PTH and the corresponding ionized calcium levels. The setpoint was calculated as the ionized calcium value corresponding to the mid percentage PTH value between $PTH_{max}$ and $PTH_{min}$, on the linear part of the sigmoidal curve.

To test the validity of the above calculation, the obtained secretory parameter values were entered in the formula defining the theoretical sigmoidal curve according to Rodbard and Hutt [29] (**Appendix**). The calculated and observed values of PTH were then compared by linear regression: the $r$ value was 0.961 ($P < 0.0001$), with an intercepta value of 0.06 (not different from 0) and the slope value of 0.97 (close to unity). Furthermore, the setpoint value obtained by our method was compared with that obtained by the logit/log curve (see **Appendix** for calculation) by linear correlation analysis. The correlation coefficient was 0.921 ($P < 0.0001$), with "b" values very close to unity (0.96) and intercepta not different from 0 (0.004).

The glomerular filtration rate (GFR), calculated as described above, was expressed as corrected values for body surface area. According to the GFR values, the renal patients (RP) were divided into three groups: group RP1 (GFR >70 ml/min; $N = 6$), group RP2 (GFR between 30 and 70 ml/min; $N = 13$), group RP3 (GFR <30 ml/min; $N = 8$).

The nine control subjects (group C) were submitted to the same study protocol, with the exception of GFR assessment by $Cr^{51}$-EDTA. Their GFR was indirectly evaluated by creatinine clearance (ranging from 90 to 158 ml/min).

Electrolytes, creatinine and urea in serum and urine were measured by standard methodology (autoanalyzer, absorption spectrophotometry and flame photometry).

Serum ionized calcium was evaluated using an ICA 1 ionized calcium analyzer (Radiometer, Copenhagen, Denmark).

PTH was measured by intact-PTH immunoradiometric assay (IRMA, Nichols Institute Diagnostics, California, USA), utilizing two different polyclonal antibodies, purified by affinity chromatography, specific for 39-84 and 1-34 regions (normal values 7 to 55 pg/ml).The intra-assay coefficient of variation was 2.4% and the interassay coefficient 5.7%.

Calcitonin (CT) was analyzed by radioimmunoassay with double antibodies, in liquid phase (Nichols). This assay has been proven to be specific for monomeric CT (the active hormone) and has very low limits of detection (3 pg/ml) [30]. The intra-assay coefficient of variation was 3.3% and the interassay coefficient 5.5%, with the normal range for males 4 to 20 pg/ml.

For vitamin D metabolite determination, a 10 ml blood sample was collected with heparin, immediately centrifuged at 4°C and stored at −20°C, until the assay was performed (not later than 20 days). Plasma 25(OH)D was measured after alcoholic extraction by competitive protein binding assay for vitamin D binding protein and tritiated antigen (Nichols). The intra-assay coefficient of variation was 7.1% and the interassay coefficient 12.8% (normal range 16 to 74 ng/ml). For the $1,25(OH)_2D$ assay, after acetonitrile extraction in the presence of tritiated calcitriol as an indicator of extraction efficiency, a plasma sample was eluted on C18OH column, with isopropanolol-hexane, then was dried by nitrogen stream and measured by the radioreceptorial method (RRA, Nichols). Each sample was dosed in duplicate. The recovery for this metabolite was between 60 and 80% and each sample was corrected for its own recovery. The intra-assay coefficient of variation was 10.0% and the interassay coefficient was 14.0% (normal range 18 to 62 pg/ml).

Statistics were calculated utilizing a $t$-test for paired data, ANOVA, simple, multiple and partial regression analysis using a statistic package (BMDP) implemented on an Olivetti M 300-10 personal computer.

**Table 1.** Basal data of renal function, calcium and phosphate parameters in patients and controls (mean ± SD)

| Group | N | Age *years* | Creatinine clearance *ml/min* | GFR *ml/min/1.73 m²* | i-Ca *mmol/liter* | i-P *mmol/liter* |
|---|---|---|---|---|---|---|
| RP1 | 6 | 39.5 ± 19.2 | 110.3 ± 29[bc] | 80 ± 12[bc] | 1.26 ± 0.04 | 0.98 ± 0.23 |
| RP2 | 13 | 41.0 ± 12.1 | 63.1 ± 18.5[a] | 44.7 ± 12.7[a] | 1.27 ± 0.06 | 0.98 ± 0.24 |
| RP3 | 8 | 47.0 ± 13.6 | 25.6 ± 13.0[ad] | 16.1 ± 7.5[ad] | 1.26 ± 0.03 | 1.10 ± 0.25 |
| C | 9 | 40.3 ± 11.2 | 125.1 ± 23 | ND | 1.27 ± 0.03 | 1.11 ± 0.10 |

ND is not determined.
[a] $P < 0.001$, RP vs. C
[b] $P < 0.001$, RP1 vs. RP2
[c] $P < 0.001$, RP1 vs. RP3
[d] $P < 0.001$, RP2 vs. RP3

**Table 2.** Basal hormonal parameters in patients and controls (mean ± SD)

| Group | N | PTH *pg/ml* | $1,25(OH)_2D$ *pg/ml* | 25(OH)D *ng/ml* | CT *pg/ml* |
|---|---|---|---|---|---|
| RP1 | 6 | 22.0 ± 5.2[de] | 35.2 ± 7.2[df] | 21.3 ± 6.1 | 11.2 ± 4.7[e] |
| RP2 | 13 | 39.0 ± 13.2[a] | 27.2 ± 12.0[a] | 24.9 ± 10.5 | 13.9 ± 5.9 |
| RP3 | 8 | 142.1 ± 79.4[ch] | 14.0 ± 7.3[cg] | 19.3 ± 11.7 | 34.1 ± 27.3[ag] |
| C | 9 | 26.3 ± 12.7 | 39.9 ± 9.7 | 23.6 ± 9.3 | 11.5 ± 4.8 |

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, RP vs. C
[d] $P < 0.01$, RP1 vs. RP2
[e] $P < 0.01$, [f] $P < 0.001$, RP1 vs. RP3
[g] $P < 0.01$, [h] $P < 0.001$, RP2 vs. RP3

## Results

### *Baseline determinations*

The basal data of renal function, calcium and phosphate parameters and calciotropic hormones in the three patient groups and in controls are shown in Tables 1 and 2.

Both ionized calcium and inorganic phosphate serum concentrations were completely overlapping in the three groups of patients and controls. Basal PTH values steadily increased with GFR reduction (Fig. 1A). However, only two out of the 13 RP2 patients had clearly elevated PTH values (that is, above mean +2 SD of C group), while all RP3 patients presented basal PTH concentrations above the normal range. Serum CT levels also progressively increased with the falling of GFR values (Fig. 1B). Again, only two RP2 patients had clearly elevated basal CT levels, while five out of the eight RP3 patients had levels above the normal range. Regarding $1,25(OH)_2$ vitamin D, its values were linearly and directly related to GFR values (Fig. 2A). No major difference was evident in 25(OH)vitamin D values either between patient groups or in comparison with controls. Furthermore, no correlation was found between its levels and GFR (Fig. 2B).

The basal values of PTH (PTHb) were inversely correlated with $1,25(OH)_2$ vitamin D, by a logarithmic relationship ($\ln PTHb = 6.47 - 0.85 \ln 1,25(OH)_2$ vitamin D; $r = 0.610$, $P < 0.001$). A direct linear correlation was also found between the basal levels of PTH and CT ($PTHb = 7.42 + 3.11\ CT$; $r = 0.847$, $P < 0.0001$). No significant relationship was found between PTHb and $1,25(OH)_2$ vitamin D on the one hand and serum calcium and phosphate levels on the other. However, multiple regression analysis revealed that GFR levels only affected PTHb values, without any independent role for either calcitriol or CT.

### *Changes in ionized calcium and PTH during $Na_2$-EDTA infusions*

Infusions of $Na_2$-EDTA induced a progressive fall in serum ionized calcium concentrations in all patient groups and controls (Fig. 3A), but were without any significant difference.

Serum PTH levels, expressed as a percentage of PTH maximal values, progressively increased during the hypocalcemic tests, and the peak values were reached before 75th minute in each group (Fig. 3B). However, in the RP2 and RP3 groups the increment rate was higher than in the RP1 and C groups, with a significant difference at 15th and 30th minutes.

### *Changes in serum ionized calcium and PTH during calcium gluconate infusions*

During calcium gluconate infusions, serum ionized calcium increased progressively, without any significant difference among RP and C groups (Fig. 4A).

Conversely, PTH (expressed as a percentage of maximal values) decreased in a curvilinear fashion, again without any significant difference among the studied groups (Fig. 4B), but at time 0 when higher values were evident in the RP3 group.

### *Kinetic parameters of the calcium-PTH relation curve*

When the results of both hypo- and hypercalcemic tests were plotted together, the PTH changes in relation to ionized calcium concentrations described the well-known sigmoid-shaped curve in each group (Fig. 5).

The values of the PTH secretory parameters are shown in Table 3. $PTH_{max}$ and, to a lesser extent, $PTH_{min}$ steadily increased with the reduction in GFR. Sensitivity values also, even if to a lesser extent, progressively increased with GFR reduction. On the other hand, setpoint values were not different in the RP groups when compared with C.

The ratio between basal and maximal stimulated levels of PTH also appeared to be increased in the RP3 group when compared with the other groups.

Next we considered the interrelationships between each of the secretory parameters and potentially affecting factors, namely, GFR, calcitriol, CT, iCa and iP. The correlation matrix is shown in Table 4. The $PTH_{max}$ was inversely related to both GFR and calcitriol values and directly to basal CT levels. However, when a multiple stepwise regression analysis was performed with $PTH_{max}$ as the dependent variable and GFR, calcitriol and CT as independent variables, GFR only appeared independently related to $PTH_{max}$ levels (Table 5).

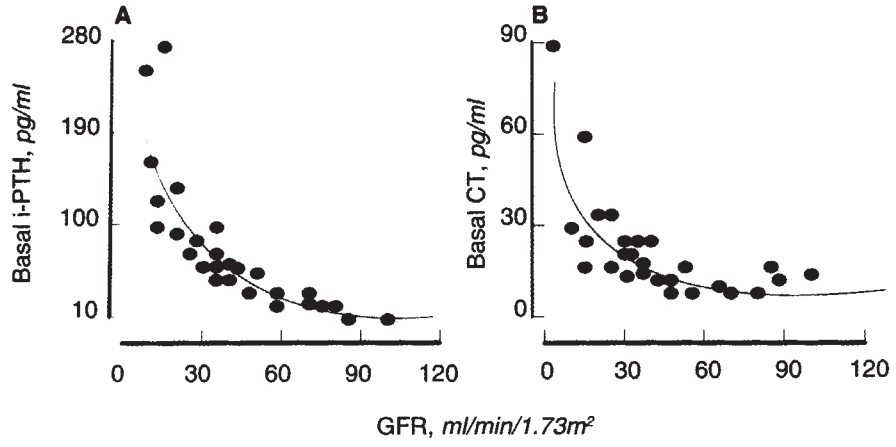


**Fig. 1.** *Correlation between basal concentrations of PTH* (**A**) *and CT* (**B**) *and GFR values, corrected by BSA.* Both parameters were significantly related to GFR by a logarithmic inverse relationship (lnPTH = 7.4 − 1.0 lnGFR: $r = 0.901$, $P < 0.001$; lnCT = 5.1 − 0.68 lnGFR: $r = 0.720$, $P < 0.001$).

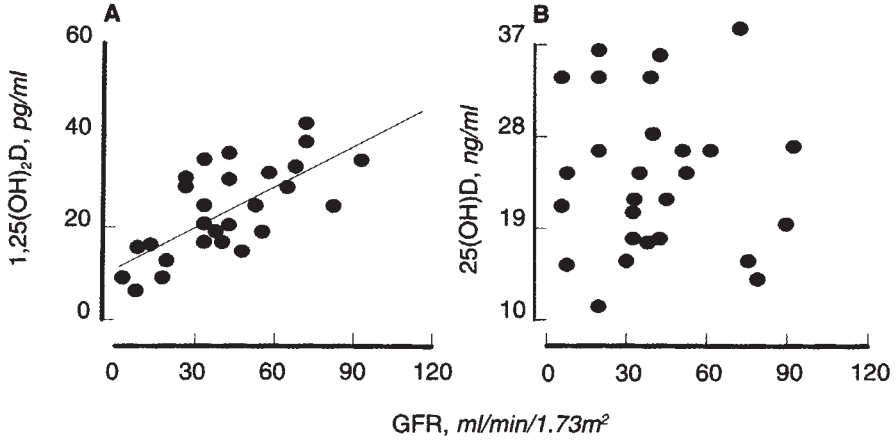


**Fig. 2.** *Correlation between GFR values, corrected for BSA, and 1,25(OH)$_2$ vitamin D* (**A**) *and 25(OH)vitamin D* (**B**) *concentrations. The calcitriol levels were inversely correlated to GFR levels, by a linear relationship [1,25(OH)$_2$D = 10.7 + 0.34 GFR; $r = 0.720$, $P < 0.001$].* No correlation was found between 25(OH)D and GFR.

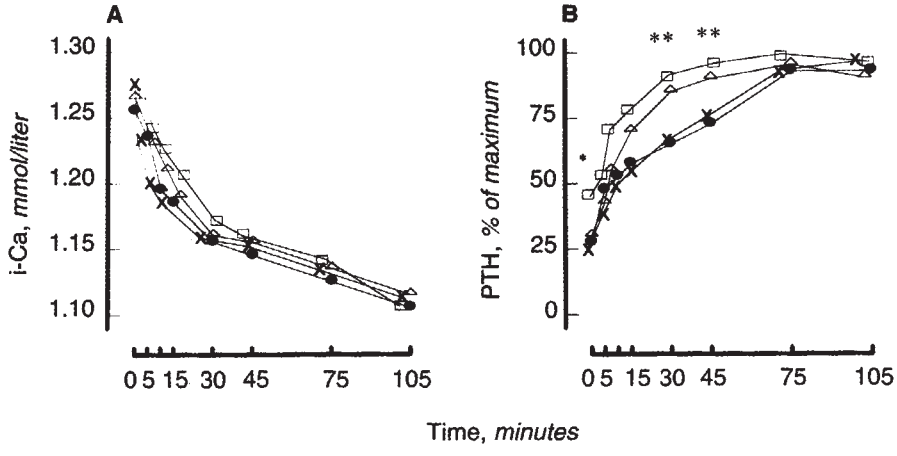


**Fig. 3.** **A.** *ionized calcium levels during Na$_2$-EDTA infusions in RP and C are depicted.* The symbols represent mean values. No difference is evident. **B.** PTH values as a percentage of maximum during the hypocalcemic test are shown; higher values are evident at 0, 15 and 30 minutes in the RP2 and RP3 groups as compared to both the RP1 and C (*$P < 0.05$; **$P < 0.01$). Symbols are: (x) C; (●) RP1; (△) RP2; (□) RP3.

No significant correlation was evident between $PTH_{max}$ and both iCa and iP.

$PTH_{min}$ values were also inversely correlated with GFR and 1,25(OH)$_2$ vitamin D levels and directly with CT, with no significant correlation with iCa and iP. Again, multiple stepwise analysis demonstrated that only GFR was independently related to $PTH_{min}$ levels (overall $r = 0.712$, $P < 0.001$; $P$ value for GFR = 0.017).

As regards sensitivity values, a weakly significant correlation was present only with GFR values.

Regarding the setpoint, no significant correlation was found with GFR, vitamin D and CT. On the contrary, a significant direct

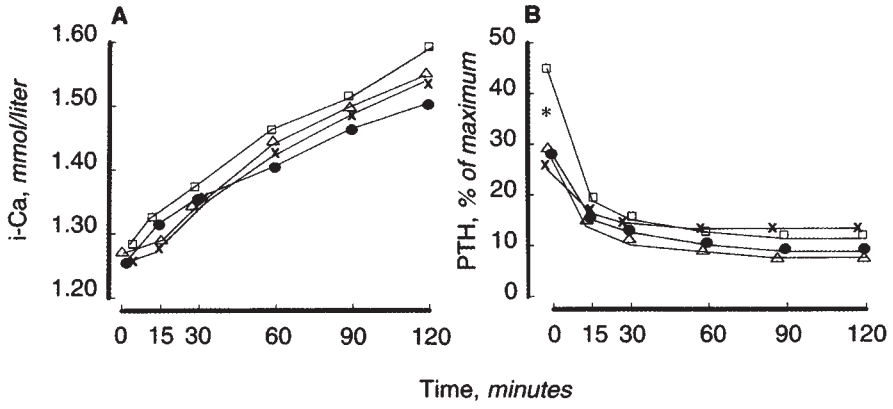


**Fig. 4.** *Ionized calcium levels (A) and PTH percent values (B) during calcium gluconate infusion in RP and C.* No statistically significant difference was observed except for basal values of PTH in the RP3 group (*$P < 0.05$). Symbols are: (x) C; (●) RPI; (△) RP2; (□) RP3.

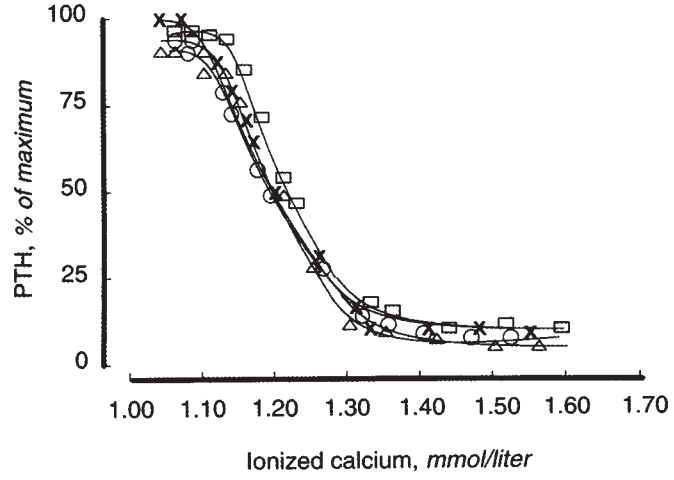


**Fig. 5.** *The sigmoidal relationships between serum ionized calcium and PTH (percent of maximum) in C and RP were obtained plotting together the results of both hypo- and hypercalcemic tests.* The symbols represent mean values, and are: (x) C; (●) RP1; (△) RP2; (□) RP3.

relationship with iP and a closer one with iCa were evident. Multiple stepwise analysis indicated iCa as the only independent variable related to setpoint levels (individual $P$ value <0.0002; overall $r = 0.741$, and $P < 0.0001$).

The ratio between basal and maximal PTH values also appeared inversely related to both GFR and $1,25(OH)_2$ vitamin D levels and directly to CT basal concentrations. In this case also, the GFR levels were the only independently related variable when tested by multiple regression analysis (individual $P$ value = 0.02; overall $r = 0.632$, and $P < 0.01$).

Finally, CT levels consistently fell after the hypocalcemic test, from a mean value of 18.7 ± 17, to 11.5 ± 7.4 pg/ml ($P < 0.001$). The CT reduction was not different in the three groups of patients (RP1 5.6 ± 4.7; RP2 4.7 ± 3.3; RP3 8.1 ± 6.7; $P = 0.35$).

## Discussion

Secondary hyperparathyroidism begins very early in the course of CRF [1–3]. In our patients, a PTH increase was evident in all the patients with GFR values below 30 ml/min, in 2 out of 13 patients with GFR between 30 and 70 ml/min, and in no renal patients with GFR above 70 ml/min. The fall in GFR was accompanied by a reduction of calcitriol and an increase in CT levels. In our patients, the $1,25(OH)_2$ vitamin D levels were inversely related to basal PTH concentrations, as found by other authors [15]. However this correlation appeared to be completely dependent on the common influence of GFR reduction on both parameters, when tested by multiple regression analysis.

On the other hand, higher CT levels were found in our patients with more pronounced GFR reduction, even though in the absence of detectable changes in calcium concentration. This finding does not have a clear explanation. It might be that higher CT levels depend on retention of inactive forms of the hormone secondary to GFR reduction. However, this hypothesis does not seem probable because the assay we used has been proven to be specific for the monomeric active form of CT [30, 31]. Furthermore, a marked reduction of CT concentration was evident after the two hour hypocalcemic test without any difference in the three groups of patients. As a second possible explanation, it cannot be excluded that the PTH increase or some other factor connected to GFR reduction might stimulate C-cells to produce CT. Recently, Tomita and Millard found C-cell hyperplasia in 58% of thyroid glands removed from uremic patients, as compared to 36% in glands removed from patients with primary hyperparathyroidism [32]. Whatever the cause of the CT increase, it might be hypothesized that its increased serum levels contribute to skeletal resistance to PTH action, as suggested by animal studies [33, 34] and/or to direct PTH stimulation, as supported by *in vitro* studies [35].

If increased PTH secretion is a well documented finding with the progression of CRF, to our knowledge, no study has as yet been performed on the secretory parameters of PTH during stimulation and suppression tests in the pre-uremic phase of renal insufficiency, utilizing a reliable PTH assay and utilizing both hypo- and hypercalcemic tests. In the only study performed in patients with moderate renal failure [1], a carboxy terminal PTH assay was used exclusively during a hypocalcemic test, so its results cannot be compared with ours. To our knowledge, our study is the first *in vivo* assessment of the complete calcium-PTH relationship curve in the preuremic stage of CRF.

In *in vitro* studies [20, 21] performed on hyperplastic parathyroid glands from patients with secondary hyperparathyroidism due to chronic renal failure, increased setpoint values were found. The other studies performed on humans on the calcium-PTH

**Table 3.** PTH secretory parameters in patient groups and controls (mean ± SD)

| Group | N | $PTH_{max}$ | $PTH_{min}$ | Sensitivity | Setpoint | PTHb/$PTH_{max}$ |
|---|---|---|---|---|---|---|
| | | *pg/ml* | | *%/mmol* | *mmol/liter* | |
| RP1 | 6 | 80.2 ± 18[df] | 12.5 ± 7.8 | 659 ± 475[a] | 1.23 ± 0.04 | 0.28 ± 0.07[e] |
| RP2 | 13 | 138 ± 25.7[ci] | 16.2 ± 12.2[h] | 628 ± 295 | 1.24 ± 0.04 | 0.29 ± 0.09[h] |
| RP3 | 8 | 312 ± 28.8[c] | 29.3 ± 15.3[b] | 894 ± 380[b] | 1.24 ± 0.03 | 0.46 ± 0.17[a] |
| C | 9 | 87.4 ± 28.8 | 8.5 ± 3.4 | 395 ± 150 | 1.24 ± 0.04 | 0.27 ± 0.12 |

[a] $P < 0.05$, [b] $P < 0.001$, [c] $P < 0.01$, RP vs. C
[d] $P < 0.05$, RP1 vs. RP2
[e] $P < 0.05$, [f] $P < 0.01$, RP1 vs. RP3
[g] $P < 0.05$, [h] $P < 0.01$, [i] $P < 0.001$, RP2 vs. RP3

**Table 4.** Correlation matrix between the PTH secretory parameters and potentially affecting factors [the values are *r* coefficient of linear regression; (ln) = logarithmic transformation]

| | GFR | $1,25(OH)_2D$ | CT | iCa | iP |
|---|---|---|---|---|---|
| $PTH_{max}$ | −0.860 (ln)[c] | −0.580 (ln)[c] | 0.777[c] | 0.063 | 0.141 |
| $PTH_{min}$ | −0.660 (ln)[c] | −0.659 (ln)[c] | 0.500[c] | 0.089 | 0.224 |
| Sensitivity | −0.350 (ln)[a] | −0.280 | 0.014 | 0.096 | 0.246 |
| Setpoint | −0.200 | 0.280 | 0.035 | 0.703[c] | 0.434 (ln)[b] |
| PTHb/$PTH_{max}$ | −0.592 (ln)[c] | −0.390 (ln)[a] | 0.454[b] | 0.212 | 0.228 |

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$

**Table 5.** Multiple stepwise regression analysis of the independent variables GFR (Y1), CT (Y2), calcitriol (Y3) on $PTH_{max}$ values (X)

| Predictive variable | Regression coefficient | Student's *t* test | *P* Value |
|---|---|---|---|
| Y1 | −0.56 | 3.54 | 0.0019 |
| Y2 | 0.27 | 1.97 | 0.0621 |
| Y3 | −0.07 | 0.48 | 0.6382 |

$r = 0.883$; $F = 24.71$; $P < 0.0001$

relationship curve are concerned with uremic patients in dialysis treatment [23–27] when a more advanced hyperparathyroidism is present. In some of these studies a reduction of the setpoint values accompanied the reduction of PTH levels after vitamin D administration. These results suggest a role of setpoint changes in causing SHP in CRF. However, Ramirez et al [27] questioned a change in setpoint values in dialyzed patients with SHP.

Our data demonstrate a progressive increase in the values of maximal and minimal PTH values, together with moderately higher values of sensitivity in RP as compared to C, with the declining of renal function.On the other hand, no change of setpoint values was evident in RP groups showing higher PTH values.

In fact, the increase of $PTH_{max}$ with the advance of renal failure is the most clear finding of our study. The maximal secretory values of PTH are a reliable index of parathyroid gland size [36]. Therefore, it is possible to argue that in our patients, with the advance of renal failure the parathyroid gland volume steadily increased from an early stage of CRF. The progressive increase in $PTH_{max}$ was found to be affected by GFR, CT and, to a far lesser extent, calcitriol levels. In a recent study Rodriguez, Felsenfeld and Llach [14] first demonstrated that CT might modulate the PTH calcemic action in renal failure in a rat model. From our results, one could hypothesize that increased CT levels might be related in some way to increased PTH secretion. However, it is not possible to establish from our data whether the link between CT and PTH secretion is a cause-effect or both hormone levels are dependent on a common factor, which was not explored in the present study.

The $PTH_{min}$ values were also increased with GFR reduction, indicating that the suppressibility of the PTH gland is reduced with ongoing CRF. Furthermore, the ratio between basal and maximal PTH also increases with the reduction of GFR, indicating that the increased tonic basal secretion is accompanied by a decrease in secretory reserve.

The sensitivity and setpoint results deserve additional methodological consideration. Some *in vitro* [20, 21] and *in vivo* studies [24] obtained the above values from an inhibition test only, by increasing calcium concentration. The information given by this kind of calculation is quite different from that obtained by the complete calcium-PTH relation curve, which is obtained by performing both hypo- and hypercalcemic challenges (as in our study). The former defines the setpoint value as the calcium level necessary to have a half-reduction of PTH basal levels, and the latter as the calcium level needed to obtain the half-maximal stimulation of parathyroid gland secretion. It is easy to argue that the first method gives a higher setpoint and, possibly, lower sensitivity values than the second. Furthermore, the results obtained by the first methodology are often not related to the real parameters of the complete sigmoidal curve. Secondly, the hypo- and hypercalcemic tests must be separated by a time sufficient enough to allow the calcium concentration to return to basal levels before the subsequent test; this is necessary to obviate artifactuarial assessment of secretory parameters, owing to the "hysteresis" phenomenon [22, 23] which consists of a lower or higher PTH concentration for a given calcium level during recovery respectively from hypo- or hypercalcemia. In our study, calcium concentrations before the second test always returned to basal levels. Finally, the computation methodology of secretory parameters might introduce some further difficulties in comparing the results from different studies, as pointed out by Fensenfeld and Llach [22]. The methodology of computation of setpoint values we used was quite close to the original mathematical model suggested by Rodbard and Hutt, as demonstrated by the close relationship between the setpoint values calculated by our method and those obtained by logit/log transformation.

The sensitivity values we found were slightly, but significantly,

increased in all RP groups, even in the RP1 patients where no detectable change in PTH levels was evident. However, no relationship was found between sensitivity and potential causal factors of SHP, excluding GFR values. Since sensitivity is expressed as a percentage change in PTH, this parameter should reflect PTH production per unitary volume of the parathyroid glands. This finding, together with the steady increase of PTH maximal levels, suggests that from the early reduction of the GFR an increased secretion rate per parathyroid cell and a progressive increase in glandular mass are the first initiating events in the beginning and development of SHP; this is without any apparent link with known causative factors, but exclusively depends on GFR reduction.

A further clear finding of our study was that no real change of setpoint values was evident with the progression of CRF and the early development of SHP. This finding is in contrast with the hypothesis of a causative role of setpoint change in the pathogenesis of SHP.

Indeed, from our data we cannot argue for a setpoint modification as a primary event in the pathogenesis of SHP in CRF. However, it cannot be excluded that in more advanced phases of SHP, in the course of CRF the setpoint might change, justifying changes in the serum calcium concentration.

In conclusion, our study demonstrates that: (1) SHP begins early with the progression of CRF; (2) the increase of PTH secretion, at least in this stage of CRF, is mainly characterized by increased secretion capacity without any change of setpoint, and this finding is consistent with an early hyperplastic change, but not with derangement in calcium concentration control by parathyroid glands; and (3) in addition to the already known factors, increased calcitonin levels seem to be, in an as yet not clear way, related to PTH hypersecretion.

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## Appendix

According to Rodbard and Hutt [29], a sigmoidal curve is described by the following four parameter logistic function:

$$Y = \frac{A - D}{1 + (X/C)^{\beta}} + D \quad (1)$$

where Y is a generic response variable, A is the upper asymptote, D the lower asymptote, X the value of independent variable expressed in log scale, C is the mid-range value; $\beta$ is the slope of the following linear regression:

$$Y' = \alpha + \beta X' \quad (2)$$

where

$$Y' = \text{logit}(Y) \quad (3)$$

$$\text{logit}(Y) = \ln(Y/[1 - Y]) \quad (4)$$

$$Y = (Y - D)/(A - D) \quad (5)$$

and

$$X' = \ln(X) \quad (6)$$

If Y, X, A and D values are known, the C term, which represents the X′ value corresponding to Y′ = 0, is easily obtained by equation (2):

$$\ln C = \alpha/\beta \quad (7)$$

Utilizing the above formulas for calculation of the setpoint value of the PTH secretion curve, one can utilize the following identities:

Y = PTH values, expressed as a fraction of $PTH_{max}$
X = ionized calcium concentration
A = $PTH_{max}$, for definition equal to unity
D = $PTH_{min}$, as fraction of $PTH_{max}$
C = Setpoint value, that is, ionized calcium concentration corresponding to the mid-range value of PTH.

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