Altered Instantaneous and Calcium-Modulated Oscillatory PTH Secretion Patterns in Patients with Secondary Hyperparathyroidism

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Abstract. The relative contributions of increased parathyroid cell mass and altered control mechanisms of parathyroid hormone (PTH) secretion in secondary hyperparathyroidism are still controversial. In this study, endogenous pulsatile PTH secretion was analyzed by the multiparameter deconvolution technique to differentiate alterations in cell mass-dependent (PTH burst mass) and regulation-dependent (frequency, synchrony, calcium responsiveness) PTH release in uremic patients. PTH concentration versus time profiles were obtained in 13 uremic and 16 healthy adults under baseline conditions and during acute hypo- and hypercalcemia. Plasma PTH half-life was increased in patients compared with control subjects (4.7 ± 1.9 versus 2.6 ± 0.1 min, P < 0.005). The baseline PTH secretion rate was elevated eightfold in the patients as a result of an increased PTH mass secreted per burst (17.1 ± 4.7 versus 2.0 ± 0.4 pM, P = 0.0001), higher burst frequency (8.0 ± 0.3 versus 6.8 ± 0.3 h⁻¹, P < 0.01), and a higher tonic secretion rate (343 ± 99 versus 30 ± 4 pM/h, P = 0.0001). Acute hypocalcemia elicited an immediate, frequency- and amplitude-mediated selective increase in the pulsatile secretory component, which was fractionally weaker in patients (+595%) than control subjects (+1755%, P < 0.001). The acceleration and the amplification of PTH bursts were 35 and 60% lower in the patient group. Acute hypercalcemia suppressed total PTH secretion by 79% in control subjects but only by 63% in patients (P < 0.002). PTH burst frequency was reduced during hypercalcemia by 30% in control subjects, but remained unchanged in patients. In conclusion, uremic hyperparathyroidism is mediated by a marked increase in glandular secretion, but also by reduced PTH elimination. The increased spontaneous PTH burst frequency and the blunted responsiveness to changes in Ca²⁺ indicate partial uncoupling of hyperplastic parathyroid glands from the physiologic regulatory mechanisms that direct pulsatile PTH release.

Recent research has provided evidence that the secretion of parathyroid hormone (PTH) in humans has a dynamic structure characterized by intermittent pulses superimposed on a tonic or basal mode of hormone release (1,2). We have previously demonstrated that the pulsatile secretory component, accounting for 25 to 30% of total PTH release, may be selectively activated as part of the parathyroid glandular immediate response to acute changes in ambient Ca²⁺ concentrations (2). This coordinated response involves modulation of the mass, the frequency, and the degree of regularity of PTH pulses. Alterations of pulsatile PTH signaling may play a pathophysiologic role in disorders of calcium homeostasis, as shown in women with postmenopausal osteoporosis (3,4). Alterations of PTH pulsatility were also observed in uremic subjects by two previous investigations; however, these studies were compromised by methodologic shortcomings regarding sample frequency and the techniques of pulse analysis used (5,6).

At present, it is controversial whether the increased PTH cell mass per se (7,8) or an impaired responsiveness of the parathyroid glands to acute changes in Ca²⁺ (9–13) is the major pathophysiologic mechanism in the development of secondary hyperparathyroidism (2nd HPT). The temporal structure of spontaneous PTH secretion in uremia is unknown, and previous investigations were not designed to evaluate the minute-to-minute dynamics of Ca²⁺-modulated pulsatile PTH release. Moreover, PTH is a peptide hormone cleared from the circulation to a major extent via the kidney (14). PTH secretion studies in uremic subjects therefore face the confounding effect of variably prolonged PTH half-life.

The study of pulsatile PTH secretion may be used as a paradigm to differentiate alterations in cell mass-dependent (PTH burst mass) and regulation-dependent (frequency, synchrony, calcium responsiveness) PTH release in uremic patients. Here, we investigated the temporal organization of spon-
taneous and Ca$^{2+}$-modulated PTH release in patients with 2$^\circ$ HPT by performing frequent serial blood sampling under unstimulated conditions and during acute hypo- and hypercalcemia induced by calcium or citrate clamp infusions. The relative contribution of tonic and pulsatile PTH release as well as of the individual kinetics of PTH removal were investigated by application of the multiparameter deconvolution technique (15) supplemented by a direct measurement of subject-specific plasma PTH disappearance half-life. Furthermore, we assessed possible alterations of the process regularity of PTH secretion by the novel Approximate Entropy statistic (16).

Materials and Methods

Study Subjects

Sixteen healthy adults and 13 patients with different degrees of chronic renal failure (CRF) participated in the studies. Their basic biochemical characteristics are given in Table 1. Healthy volunteers were recruited in the same age range (18 to 60 yr) as the patients, with normal calcium metabolism (see Table 1 for indices); no history of kidney, liver, cardiovascular, or other systemic disease; and lack of any acute or chronic medication use for several months before the study. Women receiving hormonal contraception were excluded, and the studies in female subjects were performed during the first half of the menstrual cycle. Renal disease was excluded by appropriate investigations, including measurements of blood urea nitrogen, serum creatinine, and creatinine clearance.

Inclusion criteria for the patients were advanced CRF (creatinine clearance < 30 ml/min per 1.73 m$^2$), hyperparathyroidism (PTH > 12 pmol/L), and absence of hepatic, systemic, or major cardiovascular disease. The underlying renal disorders included mesangiproliferative glomerulonephritis (n = 1) and other chronic glomerulonephritis (n = 3), reflux nephropathy (n = 3), IgA nephropathy (n = 2), Alport’s syndrome (n = 1), juvenile nephronophthisis (n = 1), hypoplastic kidneys (n = 1), and diabetic nephropathy (n = 1). The duration of CRF, defined as the time interval since the first documented elevation of serum creatinine > 1.3 mg/dl, ranged from 2 to 26 yr (mean 11.6). Duration of renal replacement therapy in the patients on maintenance dialysis was between 0.5 and 5.1 yr (mean 1.9). One dialysis patient received hemodialysis two times a week, and all other dialysis patients three times a week with a total dialysis time of 8 to 13.5 h, and a mean Kt/V urea of 1.11 (0.95 to 1.36).

Calcitriol treatment was stopped at least 4 wk before the study. Other chronic medication, including angiotensin-converting enzyme inhibitors (n = 10), diuretics (n = 9), Ca channel blockers (n = 6), $\alpha$-(n = 4) and $\beta$-adrenergic antagonists (n = 6), phosphate binders (n = 10), and sodium bicarbonate (n = 5), was continued in the usual dosage on all study days.

During the 2 wk preceding the investigations, all individuals were advised to ingest a diet containing the habitual calcium and phosphate content. Compliance was monitored by assessing calcium and phosphate excretion in 24-h urine collections. On the day before the study, the subjects were advised to refrain from athletic activities and to abstain from caffeine, nicotine, or alcohol. All subjects had a cardiological investigation, including electrocardiogram, before the study. The electrocardiogram was continuously monitored during calcium and citrate infusions. The protocol was approved by the local ethics committee. Written informed consent was obtained from each participant.

Study Design

The study protocol included one session in which, after a 75-min baseline period, acute hypocalcemia was induced by sodium citrate infusion over a 105-mm period, and one session in which the baseline period was followed by 105 min of calcium gluconate to achieve hypercalcemia. Twenty-nine subjects (13 patients, 16 control subjects) participated in the hypocalcemic clamp study, 21 of whom (12 patients, 9 control subjects) also performed the hypercalcemic clamp study. In those subjects who completed both protocols, the studies were performed in randomized order 1 wk apart.

All studies were performed between 10 a.m. and 1 p.m., after fasting for at least 12 h. Two cannulae were inserted into contralateral cubital veins, one for blood sampling at 1-min intervals (1 ml per draw), the other for infusion of a corresponding amount of 0.9% NaCl. The samples for determination of plasma PTH were withdrawn over an interval of less than 10 s, centrifuged immediately, and kept frozen at −70°C until assay. Ca$^{2+}$ was measured online at 10-min intervals during the 75-min baseline period, and at 5-min intervals during the subsequent periods of hypo- and hypercalcemia.

Table 1. Baseline blood chemistry of the healthy control subjects, preterminal renal failure patients, and patients on hemodialysis$^a$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Control Subjects (n = 16)</th>
<th>Preterminal Renal Failure (n = 7)</th>
<th>Hemodialysis Patients (n = 6, predialytic values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.91 (0.72 to 1.19)</td>
<td>4.4 (2.6 to 6.3)</td>
<td>11.7 (8.0 to 14.3)</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td>12 (5 to 16)</td>
<td>59 (40 to 77)</td>
<td>63 (46 to 75)</td>
</tr>
<tr>
<td>Total plasma calcium (mmol/L)</td>
<td>2.41 (2.26 to 2.57)</td>
<td>2.40 (2.05 to 2.62)</td>
<td>2.48 (2.24 to 2.77)</td>
</tr>
<tr>
<td>Serum phosphate (mmol/L)</td>
<td>0.97 (0.67 to 1.18)</td>
<td>1.43 (0.98 to 1.76)</td>
<td>1.79 (0.80 to 2.31)</td>
</tr>
<tr>
<td>Plasma intact PTH (pmol/L)</td>
<td>2.83 (1.21 to 4.96)</td>
<td>37.6 (12.5 to 88.2)</td>
<td>47.8 (16.5 to 138)</td>
</tr>
<tr>
<td>25(OH)D$_3$ (nmol/L)</td>
<td>54 (24 to 93)</td>
<td>60 (18 to 118)</td>
<td>84 (36 to 141)</td>
</tr>
<tr>
<td>1,25(OH)$_2$D$_3$ (ng/L)</td>
<td>52 (32 to 65)</td>
<td>26 (11 to 43)</td>
<td>14 (10 to 17)</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>47 (41 to 51)</td>
<td>42 (36 to 47)</td>
<td>38.4 (35 to 43)</td>
</tr>
<tr>
<td>Ccr (ml/min per 1.73 m$^2$)</td>
<td>118 (84 to 142)</td>
<td>20.3 (12.0 to 29.4)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Values are given as the mean and range. PTH, parathyroid hormone; 25(OH)D$_3$, 25 hydroxyvitamin D$_3$; 1,25(OH)$_2$D$_3$, 1,25 dihydroxyvitamin D$_3$; Ccr, creatinine clearance.
Citrato Clamp

After a baseline observation period of 75 min, hypocalcemia (0.2 mmol/L targeted decrease in $Ca^{2+}$) was induced by infusing sodium citrate at rates of 0.60 mmol/kg·h, 0.40 mmol/kg·h and 0.35 mmol/kg·h, respectively, in sequential 10-min intervals. Thereafter, steady-state hypocalcemia was maintained for 75 min by infusing 0.30 mmol/kg·h sodium citrate.

Calcium Clamp

After a baseline observation period of 75 min, hypercalcemia (0.2 mmol/L targeted increase in $Ca^{2+}$) was established by infusing calcium gluconate at a dose of 0.15 mmol/kg·h for 30 min. Steady-state hypercalcemia was subsequently maintained for 75 min by continuous infusion of 0.05 mmol/kg·h calcium gluconate.

Assays

For the measurement of intact PTH, a two-site immunoradiometric assay (Allegro®, Nichols, San Juan Capistrano, CA) was used. The assay has a sensitivity of 0.1 pmol/L and does not cross-react with any of the defined PTH fragments (17). All samples from an individual were analyzed at one time using the same assay. Every tenth sample was measured in duplicate. The duplicate measurements showed mean intra-assay coefficients of variation (CV) of 4.1% in the control subjects and 4.7% in the patients, respectively. The mean interassay CV was 5.8%. For further data analysis, each individual PTH measurement was assigned an SD value based on a power function relating within-sample SD2 to concentration as derived from all duplicate measurements.

An ion-selective electrode system was used to determine blood $Ca^{2+}$ concentrations (Ionometer 988-4; AVL, Bad Homburg, Germany). The results were corrected for pH 7.4. The mean intra-assay and interassay CV were below 1.5%.

Analysis of $Ca^{2+}$ Dynamics

Because the rate of change in PTH secretion is dependent on the velocity of change in $Ca^{2+}$ (18,19), and abnormalities in PTH responsiveness to changes in $Ca^{2+}$ have been suggested in patients with primary and 2α HPT (11,12,20,21), the blood $Ca^{2+}$ profiles induced by the hypo- and hypercalcemic interventions were analyzed separately in each individual clamp by fitting a four-parametric sigmoidal model to the observed $Ca^{2+}$ versus time series. This model is defined by the equation:

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

where $X$ is time, $Y$ is blood $Ca^{2+}$ concentration, $A$ is the upper-bound steady-state blood $Ca^{2+}$ concentration, $B$ is equivalent to the slope of the sigmoidal curve at the time point $C$, $C$ is the time when the half-maximal (hypercalcemia) or half-minimal blood $Ca^{2+}$ concentration (hypocalcemia) is reached, and $D$ is the lower-bound steady-state $Ca^{2+}$ level.

Analysis of Plasma PTH Disappearance Half-Life

Subject-specific disappearance half-lives of plasma PTH were measured during the initial phase of hypercalcemia. During this period, plasma PTH concentrations declined in an exponential manner to a lower but still detectable steady state. The incomplete suppressibility of the parathyroid glands has been demonstrated previously by Schwartz et al. (22) and our own studies (unpublished observation), in which ionized calcium levels were further increased by up to 0.4 mmol/L over the normocalcemic baseline within 30 min. To allow for the persistent residual secretion of PTH, the plasma PTH concentration time series was fitted to a decay model allowing for admixed baseline secretion, given by the equation: $C(t) = C_0 \times e^{-kt} + b$, where $k = \ln 2$ half-life and $b$ is the post-decay baseline PTH concentration.

Deconvolution Analysis

Each plasma PTH concentration profile was analyzed by a multiparameter deconvolution technique (15). The multiparameter deconvolution model assumed that the plasma PTH concentration at any given time was the result of five correlated parameters: (1) a finite number of discrete PTH secretory bursts occurring at specific times, and having (2) individual amplitudes (maximal rate of secretion attained within a burst), (3) a common half-duration (duration of an algebraically Gaussian secretory pulse at half-maximal amplitude), with pulses superimposed on a (4) basal time-invariant PTH secretory rate, and (5) a subject-specific monoexponential plasma PTH half-life. Estimates of the latter were obtained by the rate of decay of plasma PTH concentrations during continuous calcium gluconate infusion (see above and Figure 2). In those eight subjects in whom no hypercalcemic clamp study was performed, the group-specific half-life means obtained in the other subjects (see Results; control subjects: 2.6 min, conservative treatment: 3.4 min; hemodialysis: 6.6 min) were used. For all potential pulses, joint experimental nonlinear asymmetric confidence intervals of 95% versus experimental uncertainties in the data were computed to determine the inclusion of any peak in the final fit of the data (23). The fitting pathways described earlier for growth hormone and insulin were used here, after validation using computer-simulated and hormone-injected true-positive pulses (24,25).

Assuming a time-invariant plasma PTH disappearance half-life in each individual, the following parameters were estimated in each subseries (baseline normocalcemia, initial and steady-state hypocalemia, and hypercalcemia): number, locations, amplitudes, and half-duration of PTH secretory bursts, mass of hormone secreted per burst, and a non-negative maximal basal (tonic) PTH secretion rate. The pulsatile secretion rate is the product of the number of secretory events and the mass of hormone secreted per burst. The tonic hormone secretion rate reflects the maximal baseline amount of circulating hormone apparently underlying pulsatile secretory events. The total hormone secretion rate is the sum of tonic and pulsatile secretion rates.

Approximate Entropy Statistics

The scale- and model-independent approximate entropy statistic (ApEn) provides a test for regularity (orderliness) of fluctuations in a given hormone time series (26,27). This statistic evaluates the negative logarithm of the probability that any given particular length of consecutive points will be repeated within a tolerance or distance $r$ on next incremental comparison. Here, $m$ was set at 1, and $r$ at 0.2 (20%) of the PTH series SD, to normalize ApEn against different absolute input parameters will produce good statistical validity for ApEn values (26,27,29,30). ApEn values typically lie between 0 (perfectly ordered) and 2 to 3 (highly random) in this circumstance.

Because at least 50 to 70 sequential observations are required for reliable estimation of the approximate entropy in a data set sampled at high frequency, only the periods of normocalcemia and steady-state hypocalcemia were suitable for analysis.
Statistical Analyses

A repeated-measure ANOVA was performed to assess within-group (citrate clamp: baseline versus initial hypocalemia versus steady-state hypocalemia; calcium clamp: baseline versus steady-state hypercalcemia) and between-group changes (patients versus control subjects). Logarithmic transformation of the data was performed before statistical testing for those parameters that were not normally distributed as defined by the Shapiro–Wilk test. For those parameters where significant between-group differences were found, subgroup comparisons of patients on conservative treatment, patients on hemodialysis, and control subjects were made using Duncan’s test for multiple comparisons. Associations between parameters were evaluated by Spearman correlation analysis. A multivariate analysis of factors possibly influencing the variability of the relative changes in PTH secretion parameters within the patient group was performed using a stepwise multiple linear regression procedure. Data are given as means \( \pm \) SEM unless indicated otherwise.

Results
Baseline Clinical Data

The patient \((n = 13)\) and control \((n = 16)\) groups were well matched with respect to age (control subjects: mean 31, range, 21 to 59 yr; patients: mean 39, range, 19 to 59 yr). The gender distribution (M/F) was 12/4 in control subjects and 12/1 in the patient group.

Plasma chemistry values are shown in Table 1. As expected, serum PTH concentrations were markedly elevated and serum phosphate levels were moderately elevated in the patient group; however, total plasma calcium was not significantly different between patients and control subjects. Also, there were no significant differences in baseline plasma PTH, calcium, or phosphate levels between patients with preterminal and those with end-stage renal failure.

Dynamics of \(\text{Ca}^{2+}\) during Clamp Studies

The clamp studies were designed to shift acutely ambient \(\text{Ca}^{2+}\) concentrations by 0.2 mmol/L above and below baseline, respectively. Representative examples of the four-parametric sigmoidal fit of hypo- and hypercalcemic clamp studies for one control subject and one patient are given in Figure 1. As shown in the bottom parts of Tables 2 and 3, this target was obtained in the control subjects within 0.02 mmol/L both for hypo- and hypercalcemia.

Figure 1. Representative samples of changes in \(\text{Ca}^{2+}\) during hypocalcemic (top panel) and hypercalcemic (bottom panel) clamp studies in a healthy control subject (left column) and a patient with end-stage renal failure (right column). The line represents the curve fitted by the four-parameter sigmoidal model.
Table 2. Response of PTH release to a hypocalcemic clamp

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Subjects (n = 16)</th>
<th>Patients (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline Period</td>
<td>Initial Hypocalcemia</td>
</tr>
<tr>
<td>No. of PTH secretory bursts (h⁻¹)</td>
<td>7.04 ± 0.25</td>
<td>13.7 ± 0.59ᵇ</td>
</tr>
<tr>
<td>Mass of PTH secreted per burst (pmol/L)</td>
<td>1.61 ± 0.25</td>
<td>11.1 ± 1.49ᵇ</td>
</tr>
<tr>
<td>Pulsatile PTH secretion rate (pmol/L · h)</td>
<td>11.6 ± 1.90</td>
<td>152 ± 23.2ᵇ</td>
</tr>
<tr>
<td>Tonic PTH secretion rate (pmol/L · h)</td>
<td>29.8 ± 4.31</td>
<td>76.6 ± 36.4ᵇ</td>
</tr>
<tr>
<td>(% of total secretion)</td>
<td>(73.4 ± 3.38)</td>
<td>(25.8 ± 4.67)ᵇ</td>
</tr>
<tr>
<td>Total PTH secretion rate (pmol/L · h)</td>
<td>40.6 ± 5.16</td>
<td>231 ± 41.1ᵇ</td>
</tr>
<tr>
<td>PTH mean (pmol/L)</td>
<td>2.75 ± 0.24</td>
<td>12.1 ± 1.29ᵇ</td>
</tr>
<tr>
<td>ApEn</td>
<td>1.35 ± 0.09</td>
<td>1.22 ± 0.07ᵇ</td>
</tr>
<tr>
<td>Ca²⁺ (mmol/L)</td>
<td>1.24 ± 0.01</td>
<td>1.05 ± 0.01ᵇ</td>
</tr>
<tr>
<td>ΔCa²⁺ (mmol/L)</td>
<td>0.19 ± 0.01</td>
<td>1.53 ± 0.06ᵇ</td>
</tr>
<tr>
<td>Half-maximal Ca²⁺ response time (min)</td>
<td>17.4 ± 1.96</td>
<td>1.48 ± 0.03ᵇ</td>
</tr>
<tr>
<td>Slope</td>
<td>12.8 ± 1.50</td>
<td>28.8 ± 7.33ᵈ</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. ApEn, approximate entropy statistic. ᵇ P < 0.05, significant difference compared with the baseline period. ᵜ P < 0.05, significant difference compared with the initial period of hypocalcemia within the patient and control group, respectively. ᵆ P < 0.05, significant difference compared with control subjects.

Table 3. Response of PTH release to a hypercalcemic clamp

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Subjects (n = 9)</th>
<th>Patients (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline Period</td>
<td>Steady-State Hypercalcemia</td>
</tr>
<tr>
<td>No. of PTH secretory bursts (h⁻¹)</td>
<td>6.76 ± 0.29</td>
<td>4.65 ± 0.58ᵇ</td>
</tr>
<tr>
<td>Mass of PTH secreted per burst (pmol/L)</td>
<td>2.48 ± 0.48</td>
<td>0.48 ± 0.09ᵇ</td>
</tr>
<tr>
<td>Pulsatile PTH secretion rate (pmol/L · h)</td>
<td>17.0 ± 3.38</td>
<td>2.18 ± 0.44ᵇ</td>
</tr>
<tr>
<td>Tonic PTH secretion rate (pmol/L · h)</td>
<td>30.9 ± 3.61</td>
<td>7.60 ± 1.15ᵇ</td>
</tr>
<tr>
<td>(% of total PTH secretion)</td>
<td>(66.7 ± 4.95)</td>
<td>(76.1 ± 4.88)</td>
</tr>
<tr>
<td>Total PTH secretion rate (pmol/L · h)</td>
<td>48.0 ± 5.80</td>
<td>9.78 ± 1.35ᵇ</td>
</tr>
<tr>
<td>PTH mean (pmol/L)</td>
<td>2.95 ± 0.27</td>
<td>0.63 ± 0.06ᵇ</td>
</tr>
<tr>
<td>ApEn</td>
<td>1.47 ± 0.02</td>
<td>1.53 ± 0.06ᵇ</td>
</tr>
<tr>
<td>Ca²⁺ (mmol/L)</td>
<td>1.24 ± 0.01</td>
<td>1.46 ± 0.01ᵇ</td>
</tr>
<tr>
<td>ΔCa²⁺ (mmol/L)</td>
<td>0.22 ± 0.01</td>
<td>1.38 ± 0.02ᶜ</td>
</tr>
<tr>
<td>Half-maximal Ca²⁺ response time (min)</td>
<td>17.4 ± 1.33</td>
<td>17.8 ± 1.31</td>
</tr>
<tr>
<td>Slope</td>
<td>12.2 ± 0.94</td>
<td>21.0 ± 6.18</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Abbreviations as in Tables 1 and 2. ᵇ P < 0.05, significant difference compared with the baseline within the group. ᵅ P < 0.05, significant difference compared with control subjects.

In the patient group, the absolute decrease in Ca²⁺ during the citrate clamp study was less (0.16 mmol/L) than in control subjects (Table 2), whereas the increase in Ca²⁺ during the calcium clamp did not differ from the control subjects (Table 3). The dynamics of Ca²⁺ resetting during hypercalcemia were similar in both groups, whereas the steepness of the sigmoidal Ca²⁺ curve was significantly greater, and the half-maximal Ca²⁺ response time significantly shorter in hypercalcemia.
the patients compared with control subjects during the hypocalcemic clamp. The abnormal Ca\(^2+\) response pattern tended to be more pronounced in the hemodialyzed patients (slope 34.8 ± 15.8, half-maximal response time: 8.2 ± 2.8 min) than in the patients with preterminal CRF (slope 23.7 ± 5.5, half-maximal response time 13.4 ± 1.8 min). During the hypercalcemic clamp study, hemodialyzed patients revealed a steeper Ca\(^2+\) slope (35.8 ± 12.9) compared with the preterminal CRF patients (10.4 ± 1.6, \(P < 0.05\)), whereas the latter did not differ from control subjects.

The more rapid Ca\(^2+\) resetting during hypocalcemia in the patients may be related to their lower serum albumin levels (Table 1). Because serum albumin represents the main rapidly accessible buffer of circulating calcium, a lower albumin pool may predispose to an accelerated decrease in Ca\(^2+\) during citrate infusion. In concordance with this possibility, the Ca\(^2+\) slope was inversely \((r = -0.42, P = 0.02)\), and the half-maximal response time positively \((r = 0.63, P = 0.0002)\) correlated with serum albumin. In contrast, the characteristics of calcium dynamics were not correlated with serum phosphate or GFR.

**Plasma PTH Disappearance Half-Life**

Mean plasma half-life in patients with preterminal renal failure was only slightly increased compared with control subjects (3.38 ± 0.3 min \(versus\) 2.63 ± 0.14 min), whereas a two- to threefold elevation was observed in the end-stage renal failure patients (6.59 ± 0.76 min, \(P < 0.05\) \(versus\) control subjects). Plasma PTH half-life was inversely correlated with creatinine clearance as an estimate of GFR in both patients \((r = -0.77, P = 0.005)\) and control subjects \((r = -0.76, P = 0.02)\) (Figure 2). When all patients combined were considered, a significant inverse relationship between creatinine clearance and PTH half-life was confirmed \((r = -0.85, P < 0.001)\). Stepwise multiple regression analysis disclosed that PTH half-life accounted for 23% of the total variance of mean baseline plasma PTH, with no significant difference between patients and control subjects.

**Spontaneous PTH Secretion**

The baseline periods of the clamp studies (Tables 2 and 3) provide information about the characteristics of spontaneous PTH secretion in patients with 2° HPT in comparison to healthy subjects. On the basis of the average of the two baseline studies, spontaneous secretory bursts occurred at a higher frequency (by 15%) in the CRF patients compared with control subjects \((8.0 ± 0.3 \text{ versus} 6.8 ± 0.3 \text{ h}^{-1}, P < 0.01)\). The mean mass of PTH secreted per burst was increased eightfold in patients \(versus\) control subjects \((17.1 ± 4.7 \text{ versus} 14.3 ± 2.6 \text{ pmol/L} \cdot \text{h}, P = 0.0001)\). The elevation of both burst frequency and mass resulted in a 10-fold increase of the mean pulsatile PTH secretion rate in the patient group \((145 ± 44 \text{ versus} 14.3 ± 2.6 \text{ pmol/L} \cdot \text{h}, P = 0.0001)\). The increase in pulsatile PTH secretion was accompanied by a proportionate elevation of the tonic PTH secretion rate \((343 ± 99 \text{ versus} 30.4 ± 4.0 \text{ pmol/L} \cdot \text{h}, P = 0.0001)\), resulting in a similar fraction of total PTH secretion accounted for by tonic release in patients \((71.4 ± 13.8\%)\) and control subjects \((70.4 ± 13.9\%)\). The ApEn statistic revealed a similar degree of process regularity of the plasma PTH fluctuations in patients \((1.46 ± 0.04)\) and control subjects \((1.41 ± 0.06)\).

The intraindividual comparison of the two baseline studies also permitted us to assess the day-to-day reproducibility of PTH secretion. None of the differences between the two baseline investigations differed significantly from zero, indicating no systematic changes in baseline PTH release between the studies.

**PTH Secretion during Hypocalcemic Clamp Study**

The typical biphasic pattern of PTH release during citrate infusion is illustrated in Figure 3. A rapid amplification of PTH secretion was observed within a few minutes after the start of the citrate infusion. When steady-state hypocalcemia was reached, plasma PTH concentrations stabilized at a lower but still elevated level. To allow for this biphasic release pattern, PTH secretory characteristics were analyzed separately during the first 30 min of hypocalcemia induction and the subsequent 75 min of steady-state hypocalcemia (Table 2, Figure 4).

Both in patients and control subjects, the maximal stimulation of PTH release during hypocalcemia induction was characterized by a selective increase in the pulsatile component of secretion that was mediated by a combined increase in PTH burst mass and frequency. As illustrated in Figure 4, the relative increase in burst mass was lower in the patients \(+343 ± 96\%) than in control subjects \(+798 ± 130\%, P < 0.004)\), with a significant difference in increase between hemodialyzed \(+254 ± 76\%) and preterminal \(+420 ± 104\%, P < 0.05\) CRF patients. Similarly, the acceleration of burst

![Figure 2](attachment:image.png)
Figure 3. Calculated instantaneous PTH secretion rates during acute hypocalcemia induced by sodium citrate infusion in a healthy subject (top panel) and in a patient with 2° HPT due to chronic renal failure (bottom panel). Left ordinates are adapted to reflect percentage changes in secretion uniformly. Note differences in absolute secretion rates in the right ordinates.

frequency was significantly less marked in the patient (+63 ± 7.7%) than in the control group (+96 ± 9.1%, P < 0.01). These differences resulted in a much less pronounced increase in pulsatile secretion rate in the patient group, which was only one-third of that observed in the control subjects (+595 ± 135% versus 1755 ± 320%, P < 0.001). Tonic secretion rate increased significantly but to a minor degree in control subjects, and remained unaltered in patients.

During the subsequent period of steady-state hypocalcemia, burst frequency returned to the baseline level in both groups. Burst mass and pulsatile PTH secretion rate decreased, but remained significantly elevated above the baseline level in both groups. Again, patients tended to have a lower relative increase compared with baseline than the control subjects. The tonic PTH secretion rate increased above the baseline level in both groups. The relative increase of tonic secretion compared with the baseline level was significantly less pronounced in the patients than in control subjects (Figure 4). The regularity (orderliness) of PTH release increased during steady-state hypocalcemia (Table 2).
**PTH Secretion during Hypercalcemic Clamp Study**

During the calcium clamp, both the pulsatile and the tonic secretion component decreased rapidly (Table 3, Figures 5 and 6). Suppression was less marked in the patients than the control subjects for both pulsatile (−56 ± 14% versus −83 ± 5%, P < 0.04) and tonic (−59 ± 6% versus −75 ± 4% P < 0.04) PTH secretion. The reduction of the pulsatile PTH secretion component was due to a decreased mass of PTH released per burst in both groups (−57 ± 12% in patients versus −75 ± 5% in control subjects, NS), whereas burst frequency was only suppressed in the control group (−30 ± 8% versus +1 ± 5%, P < 0.004).

Within the patient group, the PTH burst mass and hence the pulsatile secretion rate were significantly less suppressible in the hemodialyzed patients (burst mass: −25.3 ± 23%; pulsatile secretion rate: −23.7 ± 29.9%) than in the patients with CRF (burst mass: −80.3 ± 4.5%, P < 0.05; pulsatile secretion rate: −79.8 ± 4.3%, P < 0.05). Stepwise multiple regression analysis disclosed that the variability of suppression of burst mass and pulsatile secretion rate within the patient group was explained in part by residual GFR (r² = 0.45/0.39), but not by baseline PTH, serum phosphate, or 1,25-(OH)₂-vitamin D₃ concentrations. ApEn, the index of orderliness of PTH release, did not change during steady-state hypercalcemia.
Figure 5. Calculated instantaneous PTH secretion rate during acute hypercalcemia induced by calcium gluconate infusion in a healthy subject (top panel) and in a patient with $2^\text{a}$ HPT due to chronic renal failure (bottom panel). Ordinates are adapted to reflect percentage changes in secretion uniformly. Note differences in absolute secretion rates.

**Effects of Possible Confounding Variables on PTH and Ca$^{2+}$ Dynamics**

The patient group included one patient with preterminal renal failure due to type II diabetes mellitus. With respect to a possible independent effect of diabetes on PTH release, the patient’s data did not differ systematically from those of the nondiabetic subjects. Likewise, the group of six subjects receiving chronic $\beta$-adrenergic antagonist medication showed similar results as the other seven patients with respect to all parameters under analysis.

**Discussion**

In this study, we were able to characterize several specific abnormalities in the temporal organization of PTH release in $2^\text{a}$ HPT. These findings are of relevance to a more comprehensive understanding of the control of PTH secretion by Ca$^{2+}$, as well as potential other, undefined mechanisms, e.g., neuronal, paracrine, and/or autocrine, in uremia.

First, our evaluation of subject-specific PTH plasma disappearance kinetics provides the first detailed analysis of endogenous intact PTH half-life and its dependence on renal function.
Figure 6. Comparison of relative changes in PTH secretory characteristics observed during acute hypercalcemia in healthy control subjects (O) and patients with 2° HPT (dotted line, mean ± SEM). Asterisks denote significant between-group differences (P < 0.05).

in humans. The inverse correlation of PTH half-life with GFR even within the normal range of renal function is analogous to previous observations concerning GH, another peptide hormone of similar molecular weight. Such hormones are freely filtered across the glomerular basement membrane, and irre- versibly degraded after uptake in the proximal tubule (31,32). Therefore, the metabolic clearance rate is a linear function of GFR (33). Because at any given distribution volume, disappearance half-life is reciprocally related to metabolic clearance rate, the observed exponential increase of half-life with decreasing GFR was expected under the assumption of free glomerular filtration of the hormone.

The observed two- to threefold elevation of intact PTH half-life in the hemodialysis patients has important implications in the interpretation of plasma PTH concentrations in 2° HPT: In end-stage renal failure, if the same amount of PTH is released from the parathyroid as in a healthy subject, plasma PTH concentration will be elevated two- to threefold above the normal range (34).

In the context of the foregoing two- to threefold impact of reduced hormone elimination, however, the main reason baseline plasma PTH concentrations are elevated in 2° HPT is a tenfold increase in the rate of PTH release from the parathyroid glands. The increase in total PTH secretion rate was explained...
in the frequency of PTH pulses. Interestingly, in patients with primary hyperparathyroidism, a proportionate increase in tonic and pulsatile PTH secretion, accounted for by a higher mass per burst, but not a higher burst frequency, has been reported (35). It is possible that increased PTH pulse frequency is specific for the 2° HPT of uremia. Although burst frequency was increased, the regularity (orderliness) of the secretory process was unchanged.

A unique feature of the parathyroid glands is their virtually instantaneous modulation of hormone release in response to changes in Ca²⁺. The calcium-sensing membrane protein has been identified, and although the intracellular mechanisms by which PTH release is modified are not yet entirely clear, the immediacy of the response strongly suggests a nongenomic pathway regulating the exocytosis of PTH-containing secretory vesicles. We have previously demonstrated that the hypocalcemia-modulated increase in PTH release is mediated by combined regulation of the mass and frequency of the secretory bursts (2). The amplification of PTH secretion depends on the rate of change in Ca²⁺ and is maximal, with the steepest decrease of the calcium curve (18,19). Here, we show that the relative increase of burst mass and frequency during a hypocalcemic stimulus was considerably lower in patients with 2° HPT compared with healthy subjects.

For the valid interpretation of the present data, one had to rule out the possibility that the hypocalcemic stimulus was weaker in the patient group. An analysis of the Ca²⁺ dynamics revealed a slightly steeper curve during the hypercalcemic clamp and a significantly steeper curve during the hypocalcemic clamp in the patient group. The latter effect was probably related to the lower serum albumin concentration in the uremic patients, providing a smaller buffer to compensate for the citrate-induced decrease in Ca²⁺. Moreover, the skeletal resistance to the calcemic action of PTH in uremia may have contributed to the more rapid decline in Ca²⁺ in the patients (36,37). Thus, in view of the rate dependence of the parathyroid response to hypocalcemia, the stimulus provided was, if anything, greater in the uremic patients.

Possible explanations for the reduced relative stimulation of PTH secretory burst mass during hypocalcemia induction in the patient group include: (1) a secretion rate nearly at the maximal secretory capacity under baseline conditions; (2) storage of relatively smaller amounts of PTH containing secretory granules per cell, at least compared with the circulating PTH pool; and/or (3) an intrinsically reduced responsiveness of the parathyroid glands to hypocalcemic stimulation in uremia. Arguments in favor of an increased proportion of actively secreting parathyroid cells due to a lengthened secretory phase and a shortened quiescent phase and of a possibly reduced amount of hormone stored per cell have been provided by several authors (11,38,39). The third possibility is supported by the recent finding of a diminished expression of the calcium membrane receptor in patients with uremic hyperparathyroidism (40,41). If the assumption is correct that the PTH burst mass is instantaneously controlled by direct signal transduction from the membrane calcium receptor to the intracellular structures controlling the exocytosis of secretory granules, then a reduced calcium receptor density may result in a diminished parathyroid responsiveness, either by a decreased number of cells recruitable for PTH release or by a reduced rate of granule exocytosis per cell.

The results obtained during the hypercalcemic clamp study, when the same patients were exposed to an opposite change in Ca²⁺, are also consistent with the possibility of defective Ca²⁺ sensing: The relative fraction of PTH release not suppressible by hypercalcemia was significantly greater in the patients than in control subjects. The lower suppressibility of PTH burst mass by Ca²⁺ in the uremic patients cannot be explained by the first two alternatives discussed above. Thus, a reduced sensitivity of the parathyroid gland is the only mechanism that plausibly explains abnormal modulation of PTH burst mass by both decreases and increases in Ca²⁺. A reduction in calcium receptor expression in uremia is an attractive candidate mechanism for this abnormality, although postreceptor defects cannot be excluded.

A multivariate regression analysis identified residual GFR as a strong predictor of PTH sensitivity. Although a recent study suggested an influence of gland size per se on the nonsuppressible portion of PTH release in 2° HPT (7), baseline PTH did not qualify as an independent predictor of PTH insensitivity in the present analysis. Similarly, 1,25-(OH)₂-vitamin D₃ and serum phosphate did not affect Ca²⁺ suppressibility independently.

The issue of whether the parathyroid sensitivity to changes in Ca²⁺ is reduced in uremia has so far been investigated only by means of “setpoint” analyses of the sigmoidal relationship between PTH and plasma Ca²⁺. A rightward shift of the Ca²⁺ setpoint was observed in some (9,11-14), but not all, studies in patients with 2° HPT (10,42,43). Our deconvolution analysis of moment-to-moment changes in actual PTH secretion rates during alterations of Ca²⁺ is a novel approach to this issue that avoids the shortcomings of conventional Ca²⁺ dynamics testing. The methodology of this study is superior to previous analyses by the use of a sufficiently high sampling frequency, allowance for alterations in PTH half-life and Ca²⁺ kinetics, and analysis of actual secretion kinetics rather than plasma PTH concentration-time profiles. The high-frequency sampling schedule applied here revealed a biphasic, nonsigmoidal secretion pattern of PTH that might have been missed by less frequent sampling intervals. Moreover, our analysis accounted for the Ca²⁺ rate dependence of PTH release.

Although reduced calcium sensitivity may readily explain the observed alterations in the modulation of PTH burst mass in uremia, the pathophysiology of the abnormal variability of PTH burst frequency during hypo- and hypercalcemia is less evident. The patients exhibited an elevated burst frequency at baseline, a relatively lower increase during hypocalcemia, and lack of suppressibility during hypercalcemia.

The physiologic basis for the pulsatile mode of PTH secretion by the four parathyroid glands is unknown, but some degree of intercellular and even interglandular coordination is
an evident prerequisite for synchronous hormone release. In view of the presence of neuronal fibers and β-adrenergic receptors on parathyroid cell surfaces (44–46), the most likely mechanism of synchronization is via the autonomic nervous system. Thus, a central nervous system “pacemaker” may exist in analogy to other neuroendocrine hormone ensembles. Recently, evidence has been presented that the calcium-sensing protein is expressed not only in the parathyroid and kidney but also in certain brain areas (47–49). If PTH secretion is indeed subject not only to local control in the parathyroid glands, but also to central nervous inputs, calcium-sensing receptors in the brain could provide a biological basis for a central modulation of PTH burst frequency in response to acute alterations of the calcium milieu. In that case, the disorders of spontaneous and Ca2+-modulated PTH burst frequency observed in the patient group would be consistent with the notion of a deficient expression of the calcium receptor not only in the parathyroid, but also in the brain in uremia. This possibility is speculative, but merits further research.

In summary, our detailed analysis of the dynamics of PTH secretion provides evidence that the increase in parathyroid cell mass is not the sole pathophysiologic mechanism underlying 2nd HPT in uremia, but is accompanied by distinct alterations of the temporal organization and calcium responsiveness of pulsatile PTH release. On the basis of our findings, further research is required to elucidate the mechanisms underlying synchronized PTH release and its dysregulation in uremia.

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