β1-Adrenergic Blockade Augments Pulsatile PTH Secretion in Humans

CLAUS P. SCHMITT,* JENNIFER OBRY,* REINHARD FENEBERG,* JOHANNES D. VELDHUIS,† OTTO MEHLS,* EBERHARD RITZ,‡ and FRANZ SCHAEFER*

Departments of *Pediatrics and †Internal Medicine, University of Heidelberg, Heidelberg, Germany; and ‡General Clinical Research Center, Mayo Medical and Graduate Schools, Rochester, Minnesota.

Abstract. Pulsatile peptide hormone secretion provides efficient control of specific end organ functions. To test the hypothesis that sympathetic neuronal activity drives synchronous pulsatile PTH release from the parathyroid glands, we investigated the acute effects of β1-adrenergic receptor blockade on PTH secretion patterns in a single-blinded study in nine healthy adults. Plasma PTH levels were determined at 1-min intervals. After a 75-min baseline period, seven subjects received a continuous intravenous infusion of the short-acting β1-adrenergic receptor blocker esmolol for 105 min. After a 30-min washout period, esmolol was infused for another 30 min. Two additional subjects were randomized to receive solvent infusions. PTH secretion characteristics were analyzed by multiparameter deconvolution analysis, and the orderliness of plasma PTH fluctuations by Approximate Entropy statistics.

Episodic peptide hormone secretion provides efficient control of hormone-receptor interaction, intracellular signaling, and specific target organ functions (1–3). PTH is released from the parathyroid glands in regular bursts occurring every 8–10 min, which are superimposed on a continuous, basal secretion component accounting for two thirds of total release (4).

Since the parathyroid glands are spatially separated, a higher regulatory mechanism coordinating the oscillatory component of secretion must be postulated. The dense autonomous innervation of the thyroid-parathyroid territory makes a common neuronal pacemaker a plausible candidate mechanism of oscillatory PTH release. Postganglionic sympathetic fibers extending from the superior cervical ganglia innervate the parathyroid glands via the external carotid nerve. Vagal fibers project via the thyroid and inferior laryngeal nerves from the dorsal motor nucleus to intraparathyroidal cholinergic ganglia. Nerve endings extend into the parathyroid cell parenchyma, where α- and β-adrenergic as well as cholinergic receptors are present (5–9).

Animal studies suggest that the autonomous innervation is of functional significance to the parathyroid glands. Hypocalcemia-induced, albeit not basal, PTH secretion is impaired early after bilateral superior cervical ganglionectomy in rats (10). After completion of Wallerian neuron degeneration, plasma PTH levels are increased, and the calcium sensitivity of the parathyroid remains altered (11). Clinical studies support a physiologic role of monoamine neurotransmitters in the regulation of PTH secretion, but were potentially confounded by indirect systemic effects of the compounds, most importantly on calcium homeostasis (10,12). In patients after total parathyroidectomy and heterotopic autotransplantation of parathyroid tissue, we observed a transient loss but later recovery of pulsatile PTH secretion, compatible with functional reinnervation of the autotransplanted tissue (13).

Taken together, the functional innervation of the parathyroids is suggestive of a neuronal pacemaker coordinating oscillatory PTH release. To further evaluate the role of the sympathetic nervous system in the generation of PTH pulsatility, we studied minute-to-minute PTH secretion during acute β1-adrenergic receptor blockade in healthy individuals. Indirect effects of β-adrenergic blockade on effectors of PTH release were ruled out by close monitoring of ionized plasma calcium, magnesium, phosphate, and catecholamines.
Materials and Methods

Subjects

Nine healthy male volunteers (mean age, 31.2 yr; range, 29–38 yr) participated in the study. None of the subjects was on any medication within the last 4 wk before the study or had any history of systemic disease. All subjects were advised to maintain their normal diet, refrain from athletic activities, and abstain from caffeine, nicotine, or alcohol at least 24 h before the study. Cardiac abnormalities were excluded by electrocardiography. During the investigation, ECG was continuously monitored. The protocol was approved by the Ethics Committee of Heidelberg University, and written informed consent was obtained from each participant.

Study Design

All investigations were performed from 9:00 a.m. to 1 p.m., after fasting for at least 12 h. Two cannulae were inserted into contralateral antecubital veins, one for blood sampling (1 ml per draw) at 1-min intervals, the other for infusion of a corresponding volume of 0.9% NaCl with esmolol (Brevibloc; Baxter, Unterschleißheim, Germany) or solvent. One milliliter of Brevibloc contains 10 mg of esmolol, 2.8 mg of sodium acetate, 0.55 mg of glacial acetic acid, sodium hydroxide, and/or hydrochloric acid as necessary to adjust the pH to between 4.5 and 5.5. All samples were withdrawn within 10 s, centrifuged immediately, and kept frozen at –70°C until assay. Care was taken to exclude confounding effects of the infusion procedure, the solvent or neuropsychological interference (i.e., anxiety-induced modulation of the sympathetic nervous system). Seven subjects received sequential infusions of physiologic saline and esmolol, and two additional subjects were randomized to receive solvent rather than esmolol alternating with saline infusions. The subjects were unaware whether and at which time periods during the study they received esmolol.

After a baseline period of 75 min, esmolol was infused at a starting dose of 500 μg/kg for 1 min followed by 50 μg/kg per min for 4 min, and subsequently increased every 5 min by 50 μg/kg per min to 200 μg/kg per min. The latter dose was maintained for 90 min. In six volunteers, a subsequent washout period of 30 min was followed by another 30-min period of esmolol infusion at a dose of 200 μg/kg per min. Solvent infusions mimicked the first two periods in one subject and all four periods in one other subject.

BP, heart rate, and ionized calcium were monitored every 5 min. To monitor catecholamine excretion, urine was collected from 2 h before the study until the end of the 75-min baseline period and again during esmolol infusion (minutes 75 to 240). Plasma catecholamines were determined at the start and at the end of the study.

Assays

PTH was measured using a first generation two-site immunoradiometric assay with a sensitivity of 0.1 pmol/L (Allégro; Nichols, San Juan Capistrano, CA) (14). This assay has some crossreactivity with a fragment containing 77 amino acids (presumably 7–84 PTH). We observed similar PTH pulsatility patterns when the same plasma concentrations profiles were measured with the Nichols IRMA and a novel 1.84-PTH specific (whole-PTH) assay (Scantibodies Laboratory, Inc., Santee, CA) (Schmitt CP, Gao P, Schaefer F; unpublished observation). All samples from one individual were measured at one time using the same assay. Every tenth sample was measured in duplicate, showing a mean intra-assay coefficient of variation (CV) of 4.2% and an inter-assay CV of 5.5%. For further data analysis, each singlet PTH measurement was assigned an SD value on the basis of the concentration-dependent power function of assay variance derived from all duplicate measurements. The samples for measurement of ionized blood calcium were collected in tubes containing calcium-titrated heparin. Measurements were performed within 1 min of collection using an ion-selective electrode system (Ionometer EH-F; Fresenius, Oberursel, Germany). The results were corrected for pH 7.4. Intra- and inter-assay CV of Ca²⁺ measurements were <1.5%. Adrenaline, noradrenaline, vanillyl mandelic acid, and dopamine were measured by HPLC.

25-OH-vitamin D was measured after acetonitrile extraction using a competitive protein-binding assay. 1,25-vitamin D was measured using a RIA after column chromatography of the serum.

Deconvolution Analysis

The plasma PTH concentration profiles obtained by 1-min sampling were analyzed by multiparameter deconvolution (15). This model assumes that plasma PTH concentrations are determined jointly by five correlated parameters: (1) a finite number of discrete 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 monoeXponential plasma PTH half-life of 2.6 min (16). To verify potential pulses, pulse amplitude was required to exceed the 95% joint experimental nonlin-
ear asymmetric confidence intervals (17). The fitting pathways used here were validated earlier for GH and insulin using computer-synthesized and hormone-injected true-positive pulses (18,19).

The following parameters were estimated under each study condition: number, locations, amplitudes, and half-duration of PTH secretory bursts; mass of hormone secreted per burst; and a nonnegative maximal basal (tonic) PTH secretion rate. The pulsatile secretion rate is the product of the number of secretory events and the mean mass of hormone secreted per burst. The tonic hormone secretion rate reflects the maximal non-pulsatile component. Total hormone secretion is the sum of tonic and pulsatile secretion.

Approximate Entropy Statistic

The scale- and model-independent approximate entropy statistic (ApEn) quantitates regularity (orderliness) of fluctuations in a given hormone time series (20,21). This measure represents the negative logarithm of the summed probability that an individual particular pattern length of m 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, which serves to normalize ApEn against different absolute PTH concentrations (22). Previous theoretical analyses and clinical applications have demonstrated that these input parameters produce good statistical validity for ApEn values (20,21,23,24). ApEn values lie between zero (perfectly ordered) and 2 to 3 (highly random). Typically more than 30 sequential observations afford reliable estimation of the ApEn.

Descriptive Statistics

Data are given as mean ± SEM, if not indicated otherwise. Intra-individual differences in PTH secretion characteristics between the first two infusion periods (esmolol versus baseline) were assessed by paired t tests for Gaussian and by Wilcoxon Sign-Rank tests for non-Gaussian data distributions. Changes in PTH secretion throughout the 4 infusion phases were assessed by repeated measure Analysis of Variance, using the CONTRAST statement in the ANOVA procedure of the SAS/STAT software to assess the significance of differences between individual phases.
Results

All subjects were in the normal range for serum electrolytes (Table 1), creatinine (mean, 0.88 mg/dl; range, 0.75–1.04 mg/dl), 25-OHD3 (mean, 25 ng/ml; 17–35 ng/ml), and 1,25-(OH)2D3 (mean, 32 ng/L; 23–39 ng/L) concentrations. Ionized calcium, magnesium, and phosphate remained stable throughout the study, suggesting that the observed changes of PTH secretion occurred independently of fluctuations of these electrolytes during esmolol infusion. Baseline BP was normal in all subjects. Systolic and diastolic BP slightly declined during the first esmolol infusion period (P < 0.05), but it remained stable at this level during the subsequent washout and the second esmolol infusion phase (Figure 1; Table 1). Concordant, albeit insignificant, changes were observed with respect to heart rate. In the two subjects receiving solvent instead of esmolol, BP also decreased slightly during the first hour of infusion and remained stable thereafter, with no change in heart rate.

Plasma catecholamine levels were not different before and after esmolol infusion (adrenaline, 1.1 ± 0.1 versus 1.1 ± 0.2 nM; noradrenaline, 6.2 ± 0.9 versus 5.8 ± 1.3 nM; dopamine, 1.6 ± 0.4 versus 1.9 ± 0.6 nM; all NS). Moreover, urinary excretion of adrenaline (2.9 ± 0.9 versus 2.6 ± 0.9 nmol/h), noradrenaline (7.6 ± 2 versus 5.9 ± 0.8 nmol/h), dopamine (0.05 ± 0.02 versus 0.12 ± 0.06 μmol/h), and vanillyl mandelic acid (1 ± 0.2 versus 1.6 ± 0.5 nmol/h) were not significantly changed by esmolol infusion, ruling out systemic counterregulation of biogenic monoamines that might have potentially influenced PTH release.

Infusion of esmolol induced an instantaneous, transient increase in plasma PTH in all subjects (Figure 2). Mean plasma PTH levels were 33% above baseline during esmolol infusion (P < 0.05). This increase was caused by a selective augmentation of the PTH mass secreted per burst (+117 ± 42%; P < 0.05) and a corresponding increase of the pulsatile PTH secretion component by 129 ± 44% (P < 0.05). Tonic PTH secretion was not affected significantly (+67 ± 41%, P = NS). Also, the generation and rhythmicity of PTH pulses as expressed by pulse frequency and Approximate Entropy scores (baseline, 1.53 ± 0.03; esmolol, 1.52 ± 0.03, NS) were not affected by esmolol infusion (Table 2; Figure 3).

During temporary cessation of esmolol infusion, plasma PTH decreased by 10 ± 2.7% (P < 0.05) and PTH burst frequency by 21 ± 5% (P < 0.05). The other secretion characteristics did not change significantly (Table 2).

The immediate rise in plasma PTH observed in all individuals during the first esmolol infusion was reproducible upon repeated exposure in four of six subjects. Mean plasma PTH increased by 38 ± 12%, burst mass by 173 ± 49%, and pulsatile secretion rate by 148 ± 42% above baseline level (P < 0.05) (Table 2). Pulse frequency increased significantly but remained 12 ± 9% below the pulse frequency observed during the first esmolol infusion (P < 0.05). Again, the non-pulsatile, tonic secretion rate did not change consistently.

No consistent changes of the PTH secretion pattern were

Table 1. BP, heart rate, and biochemistry during individual study periods

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 7)</th>
<th>Esmolol (n = 7)</th>
<th>Wash-out (n = 6)</th>
<th>Esmolol (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>124 ± 6.4</td>
<td>115 ± 7.2a</td>
<td>109 ± 5.0a</td>
<td>107 ± 8.2a</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>77 ± 4.4</td>
<td>70 ± 4.8a</td>
<td>64 ± 4.0a</td>
<td>62 ± 5.9a</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>75 ± 7.6</td>
<td>71 ± 6.0</td>
<td>69 ± 2.7</td>
<td>67 ± 2.5</td>
</tr>
<tr>
<td>Plasma Ca⁺⁺ (mM)</td>
<td>1.16 ± 0.04</td>
<td>1.17 ± 0.03</td>
<td>1.17 ± 0.05</td>
<td>1.17 ± 0.03</td>
</tr>
<tr>
<td>Serum phosphate (mM)</td>
<td>1.17 ± 0.20</td>
<td>1.17 ± 0.19</td>
<td>1.16 ± 0.25</td>
<td>1.13 ± 0.22</td>
</tr>
<tr>
<td>Serum magnesium (mM)</td>
<td>0.69 ± 0.04</td>
<td>0.69 ± 0.04</td>
<td>0.68 ± 0.11</td>
<td>0.68 ± 0.09</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

Letters indicate significant intraindividual differences to a baseline, b first esmolol infusion, c washout, and d second esmolol infusion periods (P < 0.05).
observed in the two subjects receiving solvent instead of esmolol.

**Discussion**

This study provides evidence for a modulatory role of β-adrenergic neurotransmission on PTH secretion in humans. Perturbation of the sympathetic nervous system by acute β-receptor blockade augments the size of the PTH pulses, but has only minor effects on the underlying oscillatory pattern of PTH release.

The observed stimulation of PTH secretion by β1 blockade adds to a controversial body of literature concerning the role of the sympathetic nervous system in the control of PTH secre-

![Figure 2](image1.png)

**Figure 2.** Representative plasma PTH concentration profile (mean ± SD of duplicate measurements, upper panel) and the corresponding calculated PTH secretion rate (lower panel) in a volunteer treated with esmolol (minutes 75 to 180 and minutes 210 to 240).

![Figure 3](image2.png)

**Figure 3.** Synopsis of individual, percentage changes in PTH secretion parameter during the first infusion of esmolol in seven healthy men. The mass of PTH secreted per burst and the pulsatile secretion rate significantly increased during β1-adrenergic blockade (P < 0.05 for intraindividual comparison to baseline).

**Table 2.** PTH secretion parameter in the six individuals who underwent two esmolol infusion periods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Esmolol</th>
<th>Wash-out</th>
<th>Esmolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean PTH (pmol/L)</td>
<td>3.0 ± 0.7</td>
<td>4.1 ± 1a</td>
<td>3.7 ± 1b</td>
<td>4.2 ± 1.1a</td>
</tr>
<tr>
<td>Pulse frequency (h⁻¹)</td>
<td>6.8 ± 0.3</td>
<td>7.2 ± 0.4a</td>
<td>5.7 ± 0.4a</td>
<td>6.3 ± 0.7b c</td>
</tr>
<tr>
<td>Mass of PTH per burst (pmol/l)</td>
<td>1.8 ± 0.6</td>
<td>3.1 ± 0.7a</td>
<td>3.8 ± 1.2a</td>
<td>4.5 ± 1.4a</td>
</tr>
<tr>
<td>Pulse amplitude (pmol/L · min)</td>
<td>0.36 ± 0.11</td>
<td>0.52 ± 0.11a</td>
<td>0.63 ± 0.17a</td>
<td>0.74 ± 0.2</td>
</tr>
<tr>
<td>Pulse half duration (min)</td>
<td>2.1 ± 0.6</td>
<td>2.3 ± 0.2a</td>
<td>2.4 ± 0.3</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>Pulsatile PTH secretion rate (pmol/L · h)</td>
<td>12 ± 4</td>
<td>23 ± 7a</td>
<td>22 ± 8</td>
<td>28 ± 8a</td>
</tr>
<tr>
<td>Tonic PTH secretion rate (pmol/L · h)</td>
<td>30 ± 10</td>
<td>42 ± 12</td>
<td>40 ± 7</td>
<td>45 ± 8</td>
</tr>
<tr>
<td>Total PTH secretion rate (pmol/L · h)</td>
<td>42 ± 10</td>
<td>65 ± 13a</td>
<td>62 ± 14a</td>
<td>73 ± 16a</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Letters indicate significant intraindividual differences versus a baseline, b first esmolol, c wash-out, and d second esmolol infusion periods (P < 0.05).
tion. In vitro, \( \beta \)-adrenergic receptor agonists clearly stimulate PTH release, and \( \beta \)-adrenergic antagonists inhibit agonist-induced PTH secretion by parathyroid cells (25,26). In vivo, the \( \beta \)-adrenergic agonist isoproterenol was found to stimulate (27), have no effect on (12), or even suppress PTH secretion (28). These discrepant results may have been confounded by uncontrolled changes in ambient Ca\(^{2+} \), which is modulated inversely to PTH by isoproterenol in humans (29). Non-pharmacologic studies have indicated that the sympathetic nervous system may exert a predominantly attenuating effect on the parathyroid in vivo. Chronic sympathetic denervation raises plasma PTH in rats (11). In humans, plasma PTH levels were suppressed by activation of the sympathetic nervous system via application of lower body negative pressure (30).

In this study, the ultra-short acting selective \( \beta \)1-adrenergic receptor antagonist esmolol was continuously infused to block \( \beta \)-adrenergic sympathetic input to the parathyroid in healthy humans. Since esmolol is devoid of any agonistic properties (31,32) and even minute changes in serum Ca\(^{2+} \), phosphate, or magnesium levels were ruled out by continuous close monitoring, our findings provide clear evidence that in contrast to the in vitro stimulation of PTH release by \( \beta \)-adrenergic receptor agonists, the basal \( \beta \)-adrenergic tone serves to dampen PTH secretion in vivo. The reasons for this discrepancy remain to be elucidated and may involve indirect effects of the sympathetic nervous system on other humoral or neural transmitter systems affecting PTH secretion by the parathyroids. Of interest in this context, extended \( \beta \)-adrenergic blockade has been demonstrated to increase bone mass by relieving the inhibitory tone exerted by the sympathetic nervous system on osteoblast activity (33,34). Inhibition of basal PTH secretion may constitute another mechanism by which the sympathetic nervous system suppresses bone turnover in vivo.

Our analysis of the underlying temporal structure of PTH secretion revealed an immediate increase of PTH pulse size in response to esmolol, while the frequency and regularity of pulses remained unchanged. The increase in tonic PTH release was less pronounced and did not reach statistical significance.

PTH pulse frequency dropped slightly after discontinuation of esmolol and increased again upon repeated exposure, but the precision of assessment of the post-esomebol burst frequency was limited by the short duration of the withdrawal period (30 min) and possible carryover effects from the preceding infusion of esmolol, which has a serum half-life of 9 min (35). Residual circulating concentrations and/or persistent biologic effects of esmolol may explain why we did not observe an increase in heart rate or BP and why plasma PTH levels did not return to baseline during the washout period. In line with this notion, the second exposure to esmolol resulted in concordant but less pronounced effects compared with the first infusion period. Interestingly these changes were not accompanied by changes in BP or heart rate, suggesting independence of the endocrine from the cardiovascular effects induced by esmolol.

The instantaneous augmentation of PTH burst mass during esmolol infusion suggests involvement of \( \beta \)-adrenergic receptors, or any other parathyroid cell membrane receptors acted upon indirectly by \( \beta \)-adrenergic blockade, in the regulation of the exocytosis of preformed hormone-containing granules, the likely mechanism of pulsatile PTH secretion.

The minor modulation of burst frequency and the lacking effect on PTH rhythmicity seems to argue against the existence of a superior neuronal pacemaker in the sympathetic nervous system causing or synchronizing oscillatory PTH release. However, we cannot exclude an initial increase in catecholamine levels and transmission of oscillatory signals via \( \alpha \)-adrenergic receptors (9), parasympathetic fibers, and/or local cholinergic ganglia (6, 36). Of interest in this context, investigations of the mechanisms responsible for pulsatile pancreatic insulin secretion revealed oscillatory electrical activity in intrapancreatic ganglia operating at a similar frequency as the insulin secretory oscillations by adjacent \( \beta \) cells (37). While preganglionic electrical stimulation augments insulin pulse amplitude (38), the exposure to nicotinic receptor antagonists increases pulse frequency (39). In analogy, PTH pulses may primarily be generated by oscillatory electrochemical activity in local cholinergic ganglia, with modulatory actions of the autonomous nervous system on the amount of hormone released per burst and, to a minor degree, on burst frequency.

In summary, our minute-to-minute assessment of the PTH secretion pattern clearly indicates a modulatory effect of \( \beta \)-adrenergic neurotransmission on parathyroid gland secretory activity. \( \beta \)1-adrenergic blockade stimulates PTH release by increasing the PTH mass secreted per burst. On the other hand, we obtained no evidence for an involvement of the sympathetic nervous system in the generation of PTH pulses per se, as would have been indicated by modulation of pulse frequency and orderliness.

Acknowledgments

The study was supported by a grant of the Else Kröner-Fresenius-Stiftung. We acknowledge the excellent technical assistance of Heinz-Jürgen Roth, Laboratory Group, Heidelberg. We appreciate the excellent support by Sylvia Gaenzler, Ruth Vierling, Bärbel Philippin, and Heike Hamm from the renal laboratory at Heidelberg University Children’s Hospital.

References


