Parathyroid Hormone 7-84 Induces Hypocalcemia and Inhibits the Parathyroid Hormone 1-84 Secretory Response to Hypocalcemia in Rats with Intact Parathyroid Glands

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Biologic effects of large C-terminal parathyroid hormone (PTH) fragments, opposite to those of N-terminal PTH, have been demonstrated. C-terminal PTH fragments are co-secreted with N-terminal PTH from the parathyroids. The aim of our study was to examine whether C-terminal PTH 7-84 regulates secretion of PTH 1-84 and affects the expression of genes of relevance for parathyroid function, PTH, calcium-sensing receptor (CaR), PTH type 1 receptor (PTHR1), and PTH-related peptide (PTHrP) genes in rat parathyroid glands. PTH 7-84 induced a significant decrease in plasma Ca$^{2+}$ in rats with intact parathyroid glands. Despite the reduction of plasma Ca$^{2+}$, no stimulation of PTH 1-84 secretion took place. Furthermore, the PTH 1-84 secretory response to EGTA-induced acute and severe hypocalcemia was significantly inhibited by PTH 7-84. During recovery from hypocalcemia, plasma Ca$^{2+}$ levels were significantly lower in the PTH 7-84-treated group, as compared with the vehicle group, and at the same time plasma PTH 1-84 levels were significantly suppressed. The expression of PTH, CaR, PTHRI, and PTHrP genes in the rat parathyroid glands was not affected by PTH 7-84. The peripheral metabolism of PTH 1-84 was not affected by PTH 7-84. PTH 7-84 did not cross-react with the rat bioactive PTH 1-84 assay. In normal rats with intact parathyroid glands, PTH 7-84 inhibited the PTH 1-84 secretory response to hypocalcemia and induced a significant decrease in plasma Ca$^{2+}$. These effects of PTH 7-84 on PTH 1-84 secretion and on plasma Ca$^{2+}$ levels were not associated with significant changes in PTH, PTHRI, CaR, and PTHrP gene expressions in the rat parathyroid glands. It is hypothesized that PTH 7-84 regulates PTH secretion via an autocrine/paracrine regulatory mechanism.

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Parathyroid hormone (PTH) is a polyhormone. For years, the biologic activity of the hormone was attributed to effects of N-terminal PTH 1-84, exerted through activation of the PTH type 1 receptor (PTHR1) (1). Recently, increasing evidence for biologic effects of C-terminal PTH (C-PTH) fragments has accumulated (2). The existence of a C-PTH receptor (C-PTHR) has been proposed on the basis of specific binding of C-PTH molecules to a rat parathyroid cell line (PT3) and to an osteoblast-like cell line (3). C-PTH fragments in the circulation originate partly from the peripheral metabolism of PTH, which occurs mainly in the liver and the kidney (4–6). Non–PTH 1-84 fragments account for approximately 10% of the C-PTH fragments and approximately 20% of the intact PTH immunoreactivity in plasma of normal individuals (7) and for 40 to 50% of the intact PTH immunoreactivity in uremic patients (7–10).

Both full-length and C-PTH molecules are present in the parathyroid glands (11). Several studies have shown that C-PTH fragments are secreted from the parathyroid glands together with intact PTH 1-84 (12–17). Under physiologic conditions, synthesized PTH undergoes intraglandular degradation, a process that depends on the extracellular Ca$^{2+}$ concentration (11). During hypocalcemia, the majority of the secreted PTH is full-length PTH 1-84. In contrast, during hypercalcemia, PTH is degraded intracellularly and only minimal amounts of PTH 1-84 are secreted, whereas the parathyroid cells secrete a number of C-PTH fragments (18,19). Recently, it was suggested that the non–PTH 1-84 that is measured in the supernatant of human parathyroid cells in culture constitute a family of PTH-processing fragments with an N-terminal structure that starts at amino acid position 4, 7, 8, 10, or 15. The peptide that starts at position 7 seems to be the most abundant fragment (20).

We previously proposed that N-terminal PTH might have an autoregulatory feedback effect on its own secretion (21). The PTHRI has been demonstrated in the parathyroid glands, and ligands for PTHRI (PTH-related peptide [PTHrP]) significantly enhanced, by several-fold, PTH 1-84 secretion during acute hypocalcemia in vivo in normal and uremic rats, as well as in vitro from parathyroid glands (21–23). As such, PTH might enhance its own secretion under the condition of hypocalcemia when increased PTH levels are needed. This investigation addressed in the rat the possibility for the existence of an autoregulatory effect of C-PTH on the secretion of PTH 1-84 in vivo and on the expression of genes of relevance for parathyroid function, PTH, PTHRI,
calcium-sensing receptor (CaR), and PTHrP in rat parathyroid glands.

Materials and Methods

Animals

Wistar rats that weighed 250 g were used. The experimental studies on the rats were performed in accordance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals and was approved by our institution. The experimental procedures were performed in rats that were under anesthesia with 50 μg/kg pentobarbital (Mebumal, Sygehus Apotekene, Aarhus, Denmark), administered intraperitoneally, and the rats were kept under anesthesia with further intraperitoneal injections of Mebumal intermittently until they were killed. It was shown previously that pentobarbital anesthesia, as compared with ketamine anesthesia, might raise rat intact PTH concentration without any change in serum calcium concentration and that this in dogs could be related to a decrease in hepatic clearance of intact PTH (24,25). We previously found that ketamine anesthesia resulted in less effective anesthesia in rats but decided to check the effects of the two types of anesthesia on plasma PTH and Ca²⁺ levels in a total of 16 rats at time 0 and after 1 h of anesthesia. The dose of ketamine was 200 mg/kg intramuscularly.

Peptides

The human PTH 7-84 (hPTH 7-84) and rat PTH 1-84 (rPTH 1-84) peptides were obtained from Saxon Biochemical (Hannover, Germany). These peptides first were dissolved in deionized water and then diluted with saline to the concentrations needed. The actual concentration of hormones in an autocrine/paracrine milieu is not known but is expected to be several magnitudes higher than that observed in the peripheral circulation. Moreover, 10 to 100 times molar concentration of these peptides were obtained from Saxon Biochemical (Hannover, Germany).

Experimental Protocols

Protocol A: Effect of hPTH 7-84 on Plasma Ca²⁺ and Plasma PTH 1-84 Levels in Normal Rats. A bolus of 100 μg of hPTH 7-84 in 1 ml of vehicle was given intravenously at time 0 to normal rats. Samples for determination of plasma PTH (p-PTH) and Ca²⁺ levels were obtained at time 0, and the parathyroid glands were harvested after 5 h, the rats were killed and the parathyroid glands were removed and kept at −80°C for studies on the gene expression.

- Group 1 rats received 100 μg of hPTH 7-84 in 1 ml of vehicle at time 0, and the parathyroid glands were harvested after 5 h (n = 10).
- Group 2 rats received 1 ml of vehicle at time 0, and the parathyroid glands were harvested after 5 h (n = 9).
- Group 3 rats received 100 μg of hPTH 7-84 in 1 ml of vehicle at time 0, and the parathyroid glands were harvested after 5 h (n = 9).

Protocol B: Effect of hPTH 7-84 on Low-Ca²⁺–Stimulated PTH 1-84 Secretion In Vivo. Hypocalcemia was induced by infusion for 1 h of 40 mmol/L EGTA (Sigma, St. Louis, MO), 3 ml/h through a catheter that was inserted in the femoral vein. Samples were obtained for determination of p-PTH and p-Ca²⁺ at time 0 and 1 h from a corresponding catheter in the femoral artery. The sample volume of 0.6 ml was replaced by 0.6 ml of saline.

- Group 5 rats received 100 μg of hPTH 7-84 in 1 ml of vehicle at time 0 and EGTA infusion for 1 h (n = 10).
- Group 6 rats received 1 ml of vehicle at time 0 and an EGTA infusion for 1 h (n = 9).

Protocol C: Effect of hPTH 7-84 on p-Ca²⁺ and p-PTH 1-84 Levels during Recovery from EGTA-Induced Hypocalcemia In Vivo. EGTA was infused for 1 h as described in protocol B. Samples for determination of p-PTH and p-Ca²⁺ were obtained at 2, 3, 4, and 5 h. At 5 h, the rats were killed and the parathyroid glands were removed and kept at −80°C for studies on the gene expression.

- Group 7 rats received 100 μg of hPTH 7-84 in 1 ml of vehicle at time 0 and an EGTA infusion for 1 h (n = 10).
- Group 8 rats received 1 ml of vehicle at time 0 and an EGTA infusion for 1 h (n = 9).

Protocol D: Effect of hPTH 7-84 on the Peripheral Metabolism of rPTH 1-84. We examined whether the PTH 7-84 fragment had an effect on the peripheral metabolism of PTH 1-84 in vivo. Because the effect of PTH 7-84 on p-PTH 1-84 levels turned out to be long lasting, we decided to check whether the metabolism of the intact hormone was affected when administered together with or at two different time points after the injection of the C-terminal fragment. Results were quantified using a standard formula for half-life (T½) and the elimination constant (Kₑ). Total clearance was calculated as dose of rPTH 1-84 divided by the area under the disappearance curves. T½ of PTH 1-84 was derived from the postdistribution part of the disappearance curve.

First, parathyroidectomy (PTX) was performed to exclude an influence of endogenous PTH. PTX was performed under microsurgery. The success of PTX later was ensured because there was no detectable PTH in plasma. One hour after PTX, the rats received a bolus of 100 μg of hPTH 7-84 in 0.5 ml of vehicle; the control rats receiving only vehicle.

- Group 9 rats underwent PTX and received 100 μg of hPTH 7-84 intravenously at 60 min after PTX. At the same time, 500 ng of rPTH 1-84 was injected intravenously and the disappearance of rPTH 1-84 was followed (n = 6).
- Group 10 rats underwent PTX and received a vehicle injection intravenously at 60 min after PTX. At the same time, 500 ng of rPTH 1-84 was injected intravenously and the disappearance of rPTH 1-84 was followed (n = 6).
- Group 11 rats underwent PTX and received 100 μg of hPTH 7-84 intravenously at 60 min after PTX. Twenty minutes later, 500 ng of rPTH 1-84 was injected intravenously and the disappearance of rPTH 1-84 was followed (n = 7).
- Group 12 rats underwent PTX and received a vehicle injection intravenously at 60 min after PTX. Twenty minutes later, 500 ng of rPTH 1-84 was injected intravenously and the disappearance of rPTH 1-84 was followed (n = 7).
- Group 13 rats underwent PTX and received 100 μg of hPTH 7-84 intravenously at 60 min after PTX. Sixty minutes later, 500 ng of rPTH 1-84 was injected intravenously and the disappearance of rPTH 1-84 was followed (n = 7).
• Group 14 rats underwent PTX and received a vehicle injection intravenously at 60 min after PTX. Sixty minutes later, 500 ng of rPTH 1-84 was injected intravenously and the disappearance of rPTH 1-84 was followed (n = 7).

Protocol E: Cross-Reactivity of C-terminal PTH fragments in the rPTH 1-84 Assay. The cross-reactivity of hPTH 7-84 in the rPTH assay was examined using seven different concentrations of hPTH 7-84 from 40 pg/ml to 40 µg/ml and of hPTH 53-84 from 16 pg/ml to 16 µg/ml. Each concentration of the two fragments was measured together with a fixed concentration of synthetic rPTH 1-84, 500 pg/ml. Quadruple determinations were performed.

Plasma Measurements
p-PTH was measured by a Rat Bioactive Intact PTH ELISA assay from Immunotopics (San Clemente, CA). This assay detects only the full-length 1-84 form of rPTH. The interassay variation of the PTH assay was 9%, and the intra-assay variation was 4%. p-Ca²⁺ was measured at actual pH by a calcium-selective electrode (Radiometer, Copenhagen, Denmark).

RNA Isolation and Reverse Transcription–PCR
Reverse transcription–PCR (RT-PCR) was used to examine the gene expression. Rat parathyroid glands and a small slice of kidney cortex (1 mm³) were obtained for total RNA extraction with Trizol (Life Technologies, Frederick, MD) after homogenization with a Polytron PT 1200CCL (Buch & Holm, Copenhagen, Denmark). Total RNA was quantified by spectrophotometry, and the quality was ensured with agarose-gel electrophoresis. First-strand cDNA was synthesized from 0.5 µg of RNA with Moloney murine leukemia virus (M-MULV) reverse transcriptase (40 U; Roche A/S, Mannheim, Germany) and random hexamer primers by incubation for 1 h at 37°C in a volume of 10 µl. Sense and antisense primers for PCR amplification of PTHRI, PTH, PTHrP, CaR, and β-actin cDNA were as follows: β-actin, sense 5'-TGTAAACCAACTGGGACGATATGGAG-3' and antisense 5'-ACAATGCGATGTCGACGATGAACA-3'; CaR, sense 5'-GGACCAGAAGGGGATCATCG-3' and antisense 5'-AAAGAGAGTGAGAGCGATTCCAAAGG-3'; PTHrP, sense 5'-GGACCAGGAAGGGGATCATCG-3' and antisense 5'-TTTCACGCTTCGCTCATAC-3'; and PTHR1, sense 5'-CTCAATGGCAATCTGCATATCTCC-3' and antisense 5'-GGCGGACAGGAGGCAAAAGG-3'.

The DNA Engine Opticon System (Merck, Boston, MA) and the QuantiTect SYBR Green PCR kit (Qiagen, Valencia, CA) were used for quantitative real-time PCR. One microliter of cDNA was submitted to amplification in a volume of 25 µl and contained 12.5 µl of 2× SYBR GREEN Mix. The sizes of the PCR products for PTTHI, PTH, PTHrP, CaR, and β-actin were 210, 244, 171, 212, and 231 bp. The reactions started with denaturation at 94°C for 30 s. Each cycle consisted of annealing at 60°C for 30 s, elongation at 72°C for 40 s, and reading of fluorescence at 78°C for 2 s. Amplifications of PTHRI and PTHrP cDNA were performed for 49 cycles; the others were performed for 39 cycles. No template control included addition of water instead of cDNA in the PCR reaction. RT control was performed by using parathyroid gland or kidney RNA, without the reverse transcriptase enzyme during cDNA synthesis. Standard curves were made by serial dilution of a pool of rat kidney cDNA (for PTHRI, CaR, and β-actin) or rat parathyroid gland cDNA (for PTH and PTHrP) to determine the relation between the time point of the log-linear increase in fluorescence signal and the initial cDNA concentration. Comparisons between samples all were based on results that were obtained within the same run. All samples were run in duplicate. The correctness of each PCR product was confirmed on 2% agarose gels and by sequencing the PCR products.

Statistical Analyses
The results are expressed as mean ± SEM. Mann-Whitney test or t test (when normal distribution was present) was used for comparison between groups. P < 0.05 was considered significant.

Results
Influence of the Type of Anesthesia on the Levels of Plasma PTH and Ca²⁺
During ketamine anesthesia, p-PTH levels were 37 ± 6 at time 0 and 40 ± 5 pg/ml at 1 h, and p-Ca²⁺ levels were 1.32 ± 0.02 at time 0 and 1.33 ± 0.01 mmol/L at 1 h. During pentobarbital anesthesia, p-PTH levels were 40 ± 16 and at 1 h were 40 ± 14 pg/ml, and p-Ca²⁺ at time 0 was 1.33 ± 0.01 and at 1 h was 1.31 ± 0.01 mmol/L (n = 8 in each group). As such, there was no significantly different effect of the type of anesthesia on either p-PTH or p-Ca²⁺ levels. Pentobarbital anesthesia therefore was chosen for these investigations.

Cross-Reactivity of C-terminal PTH Fragments in the rPTH 1-84 Assay
No cross-reactivity with hPTH 7-84 or hPTH 53-84 at seven different concentrations, from 5 × 10⁻¹² to 5 × 10⁻⁶ M, was observed in the rPTH assay.

Protocol A: Effect of hPTH 7-84 on p-Ca²⁺ and p-PTH 1-84 Levels in Normal Rats.
In the hPTH 7-84–treated rats, p-Ca²⁺ levels were significantly reduced already after 1 h (P < 0.01) and remained significantly decreased for the following 5 h of observation time (P < 0.01), as compared with the vehicle-treated group. As such, in the hPTH 7-84–treated rats, p-Ca²⁺ levels decreased progressively from a basal level of 1.33 ± 0.01 to 1.21 ± 0.02 mmol/L at 5 h (P < 0.01), whereas p-Ca²⁺ levels remained stable in the vehicle-treated group (Figure 1).

Despite significantly lower p-Ca²⁺ levels in the hPTH 7-84–treated group, p-PTH 1-84 levels did not increase but remained stable and similar to those of the vehicle-treated group for the first 2 h. Then for the following 3 h, p-PTH 1-84 levels became significantly lower in the hPTH 7-84–treated group (P < 0.05), despite the continuous fall in p-Ca²⁺ levels (Figure 1).

Protocol B: Effect of hPTH 7-84 on Low-Ca²⁺–Stimulated PTH 1-84 Secretion In Vivo
Significant acute hypocalcemia (P < 0.001) was induced by a continuous infusion of EGTA for 1 h with the same rate of reduction of p-Ca²⁺ in both experimental groups. Nadir hypocalcemia of 0.99 ± 0.02 mmol/L in the hPTH 7-84–treated group and of 1.01 ± 0.03 mmol/L in the vehicle-treated group (NS between the groups) was obtained (Figure 2).

Acute hypocalcemia induced a significant increase in p-PTH 1-84 levels in both experimental groups (P < 0.001). However, after 1 h, the secretory PTH 1-84 response to acute hypocalcemia was significantly lower in the hPTH 7-84–treated group, as compared with the vehicle-treated group (148 ± 29 versus 258 ± 29 pg/ml; P < 0.005; Figure 2).
**Protocol C: Effect of hPTH 7-84 on PTH 1-84 Secretion during Recovery from EGTA-Induced Hypocalcemia In Vivo.**

$p$-Ca$_{2+}$ levels recovered from acute EGTA-induced hypocalcemia to $1.25 \pm 0.01$ mmol/L in the hPTH 7-84–treated group and to $1.29 \pm 0.01$ mmol/L in the vehicle-treated group within the first hour after stopping the EGTA infusion. The recovery of p-Ca$_{2+}$ was significantly lower in the hPTH 7-84–treated group ($P < 0.05$), and p-Ca$_{2+}$ remained significantly lower in this group, as compared with the vehicle-treated group, for the following 4 h ($P < 0.05$; Figure 3).

**Protocol D: Effect of hPTH 7-84 on the Peripheral Metabolism of rPTH 1-84**

The rate of disappearance of exogenous rPTH 1-84 in plasma of parathyroidectomized rats is presented in Figure 4. There was no effect of hPTH 7-84 on the peripheral metabolism of PTH 1-84, irrespective of whether PTH 1-84 was administered immediately after administration of PTH 7-84 or 20 or 60 min after administration of PTH 7-84. Therefore, time 0 in Figure 4 depicts the time of administration of rPTH 1-84 in the three different protocols. The disappearance of rPTH 1-84 was followed for 37 min. Table 1 shows the results of calculations of the area under the disappearance curves, total clearance, and $T_{1/2}$ of PTH 1-84 disappearance curves. There was no significant difference between PTH 7-84– and vehicle-treated groups for any of the parameters.
The disappearance of rPTH 1-84 was not affected by hPTH 7-84 min after the injection of the peptide and then at every 4 min. A bolus of 100 µg of hPTH 7-84 or vehicle was injected intravenously into rats that underwent parathyroidectomy (PTX) at time 0. Synthetic rPTH 1-84 (500 ng) was injected intravenously at times 0, 20, or 60 min later. The disappearance of synthetic rPTH 1-84 was followed at 1 min after the injection of the peptide and then at every 4 min. The disappearance of rPTH 1-84 was not affected by hPTH 7-84 (NS). n = 7 in each group.

Figure 4. Effect of hPTH 7-84 on the peripheral metabolism of rat PTH 1-84 (rPTH 1-84). A bolus of 100 µg of hPTH 7-84 or vehicle was injected intravenously into rats that underwent parathyroidectomy (PTX) at time 0. Synthetic rPTH 1-84 (500 ng) was injected intravenously at times 0, 20, or 60 min later. The disappearance of synthetic rPTH 1-84 was followed at 1 min after the injection of the peptide and then at every 4 min. The disappearance of rPTH 1-84 was not affected by hPTH 7-84 (NS). n = 7 in each group.

Effect of hPTH 7-84 on the Gene Expressions of PTH, PTHR1, CaR, and PTHrP in Rat Parathyroid Glands

PTH 7-84 had no significant effect on the levels of PTH, PTHR1, CaR, or PTHrP mRNA in parathyroid glands at either 1 h (Figure 5) or 5 h (Figure 6) after the administration. Similarly, no differences in mRNA expressions in parathyroid glands were detected between hPTH 7-84– or vehicle-treated groups, in which acute hypocalcemia was induced by EGTA infusion (data not shown).

Discussion

In our in vivo study, it was shown clearly that hPTH 7-84 significantly inhibited the PTH 1-84 secretory response to acute hypocalcemia in the rat. Furthermore, a hypocalcemic effect of PTH 7-84 was demonstrated in rats with intact parathyroid glands, and this effect did not result in stimulation of PTH 1-84 levels.

A hypocalcemic effect of PTH 7-84 was shown previously by Slatopolsky et al. (27) using a similar total dose of the peptide but in PTX rats that were kept on a low-calcium diet and by Nguyen-Yamamoto et al. (28) in thyroparathyroidectomized rats. In this study, hypocalcemia was induced by PTH 7-84 despite the presence of intact parathyroid glands and despite the use of a standard calcium content in the diet. It is interesting that hypocalcemia did not induce increased PTH 1-84 secretion in the PTH 7-84–treated rats of our study. Because it was shown previously by Slatopolsky et al. (27) that concomitant administration of PTH 1-84 and PTH 7-84 neutralized the hypocalcemic effect of PTH 7-84, the inhibitory effect of PTH 7-84 on the secretion of PTH 1-84 seems to be obligatory for induction of hypocalcemia by PTH 7-84 in intact rats. As such, our results add a novel aspect to the understanding of the parathyroid–bone axis.

Whether the effect of PTH 7-84 on PTH 1-84 secretion from the parathyroid glands is mediated through the putative C-PTH receptor remains to be shown. This receptor has not yet been cloned. Direct physical evidence of the existence of a C-PTH receptor was established by cross-linking of the 125I-Tyr34-hPTH 19-84, which does not bind to PTHR1, to 40- and 90-kD proteins in ROS17/2.8 rat osteoclastic cells, and to 90-kD protein in rat parathyroid cells (3). Furthermore, studies on clonal osteocytic cells that were isolated from fetal calvaria of PTHRI-null mice showed displacement of the same radioligand by the C-PTH molecules, hPTH 1-84, 19-84, 24-84, and 39-84, but not by N-terminal hPTH 1-34. These results suggested that a C-terminal PTH receptor might exist and might have actions independent of the presence of PTHR1 (29).

Evidence for a specific action of a C-PTH receptor on bone cells is emerging. Divieti et al. (29) found in vitro that ligands that interacted with the putative C-PTH receptor increased the rate of osteocytic cell death, whereas an antiapoptotic action of hPTH 1-34 through the PTHR1 activation in dexamethasone-stimulated osteoblastic and osteocytic cells was shown by Jilka et al. (30). These results together with our results and the previous results from Slatopolsky et al. (27) and Nguyen-Yamamoto et al. (28) showing a hypocalcemic action of PTH 7-84 suggest that PTHR1 and the putative C-terminal PTH receptor might exert opposite effects on bone. Therefore, we speculated that this also might be the case at the level of the parathyroids. The inhibitory effect of hPTH 7-84 on the secretion of PTH 1-84 shown in this study theoretically might antagonize the stimulatory effect of N-terminal PTH on its own secretion, as previously proposed by our group (21,22), by activating the two different PTH receptors.

Another theoretical possibility is that PTH 7-84 regulates the endocytosis of PTHR1 in the parathyroid cells and thereby decreases the amount of receptors that are available for the binding of N-terminal PTH and in this way diminish the auto-stimulatory action of PTH 1-84 on its own secretion. Such a regulatory effect of C-terminal PTH fragments on endocytosis of PTHR1 was shown previously (31). PTH 7-84 does not bind to the PTHR1 in kidney-derived cells or in osteoblast-like cells (3,28,32). Recent studies, however, have demonstrated that PTH 7-34 and PTH 7-84 induced endocytosis of PTHR1 in kidney distal tubular cells and rat osteosarcoma cells, which lack the adaptor protein Na/H exchange regulatory factor 1 (31). This novel finding raises the intriguing possibility that PTH 7-84 might antagonize the actions of PTHR1 agonist in a cell-specific pattern and that PTHR1 activation and endocytosis can be dissociated. We showed previously that PTHrP 1-40, a ligand for PTHR1, enhanced the low-Ca2+-stimulated PTH secretion in vivo, and in vitro in the rat parathyroid glands, and it was shown further that PTHrP mRNA was expressed in rat parathyroid glands (21,22). This result showing an inhibitory effect of PTH 7-84 on PTH secretion in hypocalcemia might be mediated by hPTH 7-84–induced PTHR1 endocytosis in the parathyroid glands. However, the mechanism of the inhibition of PTH 1-84 secretion by PTH 7-84 remains to be established as it also remains to be shown that this is a direct effect on the parathyroid cells. In this study, we excluded that hPTH 7-84 accelerated the clearance of the intact rPTH 1-84, because our results showed that hPTH 7-84 had no effect on the peripheral metabolism of rPTH 1-84.

β-Ca2+ might affect the peripheral metabolism of PTH (33–35). It was observed by our group in the isolated perfused liver
model that low extracellular Ca\(^{2+}\) resulted in a lower hepatic clearance of intact PTH, as compared with high Ca\(^{2+}\) (36). In our study, the p-Ca\(^{2+}\) levels were decreased significantly by hPTH 7-84 in rats and PTH levels were inhibited. Therefore, the decrease in PTH levels could not be explained by any alteration of the clearance of PTH by Ca\(^{2+}\).

The rate of clearance of PTH 7-84 has not been established in normal rats. It has been suggested that the half-life of large C-PTH fragments is longer than that of PTH 1-84 (37). This observation might partly explain the long-lasting inhibitory effect of PTH 7-84 on PTH secretion in our study. It has been shown that the type of anesthesia can affect PTH levels and the hepatic clearance of PTH (24,25). In our study, it was shown that pentobarbital anesthesia at least for the first hour of anesthesia did not influence PTH levels. The significant effect of PTH 7-84 on PTH levels during acute induction of hypocalcemia occurred, however, already at 1 h, as shown in Figure 2. The increase of PTH levels over time without any change in Ca\(^{2+}\) concentration, as observed in the control group in Figure 1, might be due to an influence of several hours of anesthesia.

The lower p-Ca\(^{2+}\) levels in PTH 7-84–treated rats did not result in an increase of PTH mRNA expression in parathyroid glands. It might be that the duration of low p-Ca\(^{2+}\) in our study was too short or the depth of hypocalcemia was too small to increase the amount of PTH mRNA, because it was shown previously that it might take several hours or days for low p-Ca\(^{2+}\) to increase PTH gene expression (38–40). Finally, PTH 7-84 might offset the increase of PTH mRNA that is induced by low Ca\(^{2+}\).

The exact mechanism of an inhibitory effect of PTH 7-84 on PTH secretion in vivo in the rat remains to be established. These results on the expression of the genes of relevance for parathyroid function are shown in Table 1. The metabolism of exogenous rPTH 1-84 in PTX rats was examined using pentobarbital anesthesia and PTH 7-84 treatment.

### Table 1. Metabolism of exogenous rPTH 1-84 in PTX rats +/− PTH 7-84\(^a\)

<table>
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<th>Minutes(^b)</th>
<th>Vehicle</th>
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<tr>
<td>0</td>
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<tr>
<td>AUC (pg/ml per min)</td>
<td>8930 ± 968</td>
<td>9767 ± 1379</td>
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<tr>
<td>Total clearance (ml/min)</td>
<td>59.6 ± 6.9</td>
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<tr>
<td>Tβ (min)</td>
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<td>4.95</td>
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<td>20</td>
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<td>AUC (pg/ml per min)</td>
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<td>71.0 ± 5.9</td>
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<td>Tβ (min)</td>
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<td>4.97</td>
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<td>Tβ (min)</td>
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<td>4.94</td>
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</tbody>
</table>

\(^a\)AUC, area under the disappearance curve; rPTH, rat parathyroid hormone; T, half-life. NS between vehicle and PTH 7-84 groups in all experiments.

\(^b\)Time between PTH 7-84 and PTH 1-84 injection in rats that underwent parathyroidectomy.
roid function suggest that the effect of PTH 7-84 is nongenomic. As such, PTH 7-84 might affect the secretion of PTH 1-84 by directly influencing the exocytosis or by regulating other paracrine/autocrine factors of importance for PTH 1-84 secretion. Several such factors have been proposed to regulate PTH 1-84 secretion, some stimulating (N-terminal PTH and PTHrP) and others inhibiting (chromogranin A, chromogranin A–related peptides, and endothelin-1) (21,22,41–43). On the basis of these results, showing an inhibitory effect of C-terminal PTH on PTH 1-84 secretion, and our previous results, showing a stimulatory effect of PTH1R ligands on PTH 1-84 secretion by PTH fragments secreted by the parathyroid cells (21,22).

In the normal rats, the p-Ca^{2+} levels were significantly reduced by PTH 7-84 already at 1 h, and this hypocalcemic effect of hPTH 7-84 persisted for the following 5 h. In rats that received an infusion of EGTA, the decrease in Ca^{2+} levels was controlled by EGTA and was similar in PTH 7-84– and vehicle-treated rats. The recovery from hypocalcemia, however, was influenced by PTH 7-84, and the Ca^{2+} levels that were obtained were significantly lower. The mechanism might be related to lower levels of PTH in PTH 7-84–treated rats or related to a possible direct effect of PTH 7-84 on bone. As such, it has been shown that PTH 7-84 inhibited calcium release from neonatal mice calvaria, both in the basal state and when bone resorption was stimulated by PTH 1-84, PTH 1-34, calcitriol, prostaglandin E, or IL-11 and that PTH 7-84 inhibited the vitamin D–dependent formation of osteoclast-like cells in murine bone marrow cultures (26). Therefore, hPTH 7-84 could inhibit bone resorption and regulate the rate of osteoclastogenesis through the putative C-PTHR.

**Conclusion**

In normal rats that were kept on standard diet, PTH 7-84 induced a significant decrease in p-Ca^{2+} levels. This decrease in p-Ca^{2+} was associated with an inhibition of PTH 1-84 levels. PTH 7-84 suppressed the PTH 1-84 secretory response to acute EGTA-induced hypocalcemia. The inhibitory effect of PTH 7-84 on PTH 1-84 secretion was observed already at 1 h and persisted for 5 h. PTH 7-84 did not affect the peripheral metabolism of PTH 1-84. The expression of PTH, PTH1R, CaR, and PTHrP genes in the rat parathyroid glands was not influenced by PTH 7-84. PTH 7-84 might regulate PTH 1-84 secretion via a new autocrine/paracrine regulatory mechanism.

**References**

1. Juppner H: Receptors for parathyroid hormone and parathyroid hormone-related peptide: Exploration of their biological importance. **Bone** 25: 87–90, 1999