CLINICAL STUDY

Large carboxy-terminal parathyroid hormone (PTH) fragment with a relatively longer half-life than 1-84 PTH is secreted directly from the parathyroid gland in humans

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Abstract

Objective: It was discovered that an immunoreactive large carboxy-terminal parathyroid hormone (PTH) fragment (large C-PTH), likely 7-84 PTH, is present in the circulation. However, very little is known about the production and metabolism of this large C-PTH. Combining a whole molecule PTH (whole PTH) immunoradiometric assay (IRMA) specifically for 1-84 PTH and an intact PTH (iPTH) IRMA for the sum of 1-84 PTH and large C-PTH, we were able to assess the circulating level of this large C-PTH as well as the glandular secretion and metabolism of this large C-PTH in primary hyperparathyroidism (pHPT).

Methods: This study consisted of two patient groups consisting of 77 pHPT patients with a single adenoma. Of these, 43 comprised the venous sampling study group and 70 comprised the intra-operative PTH study group. (Seven patients belonged only to the former group, 34 patients to only the latter group, and 36 patients to both groups.) Preoperatively, blood samples were drawn from the bilateral internal jugular vein by ultrasonographic guidance and from the peripheral vein ($n = 43$). During surgery, blood samples were drawn after anesthesia (basal level), before excision (pre-excision level) of one enlarged parathyroid gland, and at 5, 10, and 15 min post-excision ($n = 70$).

Results: There were 26 patients whose iPTH assay levels differed by more than 10% between the right and left internal jugular. In 24 of the 26 patients, the large C-PTH levels obtained from the adenoma side were significantly higher than those from the contralateral side (117 ± 135 vs 43 ± 33 pg/ml, $P < 0.001$). The plasma whole PTH values decreased more rapidly than the iPTH values after parathyroidectomy ($P < 0.001$).

Conclusions: Our study has demonstrated that the large C-PTH, likely 7-84 PTH, is directly released from the parathyroid gland in humans. Since the half-life of 1-84 PTH is much shorter than large C-PTH, likely 7-84 PTH, it would be advantageous to use an assay that specifically measures 1-84 PTH for intra-operative monitoring of parathyroidectomy.

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Introduction

Parathyroid hormone (PTH) plays a critical role in the regulation of bone and mineral metabolism. Circulating PTH as measured by current assays has shown a high degree of immunoheterogeneity due to the occurrence and assay of cross-reactivity of various PTH fragments either from metabolism of the intact PTH within the parathyroid glands or in the peripheral tissues, such as in the liver and kidney (1–4). It has been thought that the older second generation intact PTH (iPTH) assay utilizing two distinct antibodies directed against the carboxyl- and the amino-terminal portions of the

hormone measures 1-84 PTH only (5, 6). However, Brossard *et al.* (7) and Gao *et al.* (8) reported that most commercial iPTH assays cross-react with non-1-84 PTH, a large carboxy-terminal PTH (large C-PTH) fragment which migrates under high pressure liquid chromatography (HPLC) conditions with similar hydrophobicity to that of synthetic 7-84 PTH. Recently, whole molecule PTH (whole PTH) measured by a novel immunoradiometric (IRMA) assay was shown to measure specifically 1-84 PTH exclusively (7, 9–12).

PTH stored in secretory granules is either secreted in response to the change in extracellular ionized calcium level or is intracellularly degraded. Intracellular

PTH degradation increases in states of hypercalcemia with a low PTH secretion rate but, on the contrary, it decreases in hypocalcemic conditions when PTH secretion is enhanced (13–15). Slatopolsky *et al.* (9) reported that 44.1% of total intracellular PTH of surgically excised parathyroid glands from uremic patients were the non-1-84 PTH, likely 7-84 PTH. They also found that the human 7-84 PTH fragment is not only biologically inactive as far as the production of cAMP *in vitro* in the rat osteosarcoma cell line (ROS/17.2) is concerned, but also functions with antagonistic effects against the biologically active 1-84 PTH when both 1-84 and 7-84 PTH are given in parathyroidectomized (PTX) rats (9). Moreover, the 7-84 PTH showed an inverse biological activity to 1-84 PTH resulting in hypocalcemia (9). Faugere *et al.* (16) reported that 7-84 PTH reduced bone formation rate/bone surface in thyroparathyroidectomized (TPTX)/nephrectomized rats. Although the presence of large C-PTH in the parathyroid gland has been documented, in this study the secretion of large C-PTH directly from the parathyroid gland was demonstrated by catheter-localized sampling of human plasma.

Patients and methods

Patient selection

This study consisted of two patient groups of 77 patients with primary hyperparathyroidism (pHPT) with a single adenoma. Of these, 43 comprised the venous sampling study group and 70 comprised the intra-operative PTH study group. (Seven patients belonged only to the former group, 34 patients to only the latter group, and 36 patients to both groups.) Part of the data from the intra-operative group have been reported previously (17). The clinical and biochemical data for the 77 patients are summarized in Table 1. Eight had a history of thyroidectomy for benign thyroid nodules ($n = 4$) or papillary thyroid

cancer ($n = 4$). Two patients were referred for persistent hypercalcemia after parathyroidectomy.

Preoperative localization studies were performed on all patients with ultrasonography, ^{99m}Tc sestamibi scanning and magnetic resonance imaging.

Preoperative laboratory tests

Blood and urine samples were collected after an overnight fast. Serum levels of alkaline phosphatase, total calcium, albumin and inorganic phosphate were measured by routine automated procedures. Preoperative serum iPTH was measured by a two-site immunochemiluminometric assay (18). The protocol was approved by the Noguchi Thyroid Clinic. All subjects gave written informed consent.

Blood samples before and during surgery

Preoperatively, blood samples for whole PTH and iPTH assay were drawn from the bilateral internal jugular vein by ultrasonographic guidance and from the peripheral vein. This procedure was basically one of the preoperative localization studies to facilitate a minimally invasive parathyroidectomy.

EDTA plasma samples were drawn via the peripheral arterial catheter after anesthesia (basal), before excision of one enlarged parathyroid gland, and at 5, 10 and 15 min post-excision. A quick intra-operative PTH (QPTH) assay was performed by a two-site immunochemiluminometric assay at 10 min in all cases (18). A decrease in the QPTH of 50% or more in the sample taken 10 min after excision compared with the higher value of either basal or pre-excision was used to predict successful parathyroidectomy. Part of the EDTA plasma samples were kept at -70°C until later analysis of iPTH and whole PTH.

PTH assay for stored samples

Whole PTH and iPTH IRMAs were performed on the same day. EDTA plasma samples were obtained 1 day before surgery and during parathyroidectomy. The whole PTH assay (Scantibodies Laboratories, Santee, CA, USA) was reported previously (9, 10). The whole PTH concentration of 135 normal adults (used for the reference range) was 22.7 ± 7.2 (means $\pm$ s.d.) and the 95 percentile reference range was defined from 7 to 36 pg/ml. The iPTH assay was performed using the Scantibodies total PTH IRMA kit (Scantibodies Laboratories). These two PTH IRMAs are designed and produced such that both assays share the same set of assay standards which were produced by spiking synthetic 1-84 PTH into a PTH-free human plasma matrix. Both assays also share the same production lot of anti-39-84 PTH antibody-coated beads, the same wash buffer and a similar assay procedure. The only difference between these two PTH assays is that two different

Table 1 Preoperative clinical and biochemical data for 77 pHPT patients. Values are means $\pm$ s.d.

	Value	Reference range
Age (years)	58 ± 13	
Sex (female/male)	67/10	
Serum calcium (mmol/l)	2.73 ± 0.22	2.20–2.54
Serum phosphate (mmol/dl)	1.00 ± 0.14	0.81–1.45
Serum creatinine ($\mu\text{mol/l}$)	62 ± 18	44–100
Serum BUN (mmol/l)	5.0 ± 1.4	3.2–6.8
iPTH (pg/ml)	137 ± 84	10–65
Whole PTH (pg/ml)	95 ± 61	7–36
Large C-PTH (pg/ml) ^a	41 ± 31	
Parathyroid gland weight (mg)	647 ± 761	

^a The large C-PTH was calculated by subtracting the whole PTH value from the iPTH value. BUN, blood urea nitrogen.

tracer antibodies are used, which accounts for significant different specificities in binding 1-84 PTH and N-truncated PTH fragment. In fact, the whole PTH assay measures 1-84 PTH exclusively and the iPTH assay determines the sum of 1-84 PTH and the large C-PTH as previously validated using HPLC fractionated samples (9). Large C-PTH, likely 7-84 PTH, was calculated by iPTH minus whole PTH. The identification of 7-84 PTH has been confirmed previously (7, 9, 12).

Statistical analysis

Data are expressed as means $\pm$ s.d. unless otherwise indicated. Statistical analysis was based on ANOVA for repeated measurements and a generalized linear regression analysis. The half-life time of PTH molecules was estimated by fitting an exponential reduction with an above zero baseline value: $Y(t) = (100 - b)\exp(-at) + b$, where $Y(t)$ stands for the percentage level of PTH at t min after excision of the hyper-functioning parathyroid, $Y(0) = 100$ by definition, the coefficient, a , represents the speed of PTH reduction, $a > 0$, and the parameter, b , represents the baseline level of PTH without hyper-functioning parathyroid, $0 < b < 100$. The parameters, time to 50% reduction, and their standard errors were estimated by non-linear fit platform with inverse prediction in SAS JMP, version 5.0 (SAS Institute Inc., Cary, NC, USA). For pairwise comparisons, the paired t -test was applied. Correlation was determined by calculating Pearson's correlation coefficient. $P < 0.05$ was considered statistically significant.

Results

The iPTH values versus the whole PTH are plotted in Fig. 1 for the 77 patients included in the two study

Figure 1 Regression parameters of preoperative plasma iPTH values and whole PTH in pHPT.

groups. There was a significant correlation between the iPTH values and whole PTH in the pHPT patients of this study. The percentage of large C-PTH in iPTH in peripheral plasma samples was $31 \pm 13\%$ in those patients.

In 43 patients making up the venous sampling study group, there were 26 patients whose iPTH assay levels differed by more than 10% between the right and left internal jugular. The mean difference in iPTH and whole PTH was $50 \pm 25\%$ (range 10–92%) and $51 \pm 27\%$ (range 3–96%) respectively. For 24 of the 26 patients, the level of large C-PTH obtained from the adenoma side was significantly higher than from the contralateral side and from the peripheral vein (117 ± 135 vs 43 ± 33 or 4 ± 540 pg/ml, $P < 0.001$). The proportion of whole PTH to large C-PTH was higher in the adenoma-side sample than in the peripheral vein and the contralateral side samples. The levels of iPTH, 1-84 PTH, whole PTH/large C-PTH and the percentage of large C-PTH in iPTH in plasma samples obtained from the internal jugular vein at the adenoma side were significantly different from those obtained from the contralateral side or from the peripheral vein (Table 2).

All of the 70 patients of the intra-operative study group underwent successful parathyroidectomies (over 50% reduction in QPTH assay at 10 min was confirmed in the operating room) and the patients were normocalcemic or hypocalcemic post-operatively and in follow-up studies ranging from 3 to 25 months. There were no intra-operative or post-operative permanent complications. The mean weight of a single adenoma was 647 ± 761 mg. There were significant differences in the percentage decrease between whole PTH and iPTH values after parathyroidectomy (Fig. 2,

Figure 2 Decline of whole PTH (●), iPTH (■) and large C-PTH (▲) after excision of one enlarged parathyroid gland in 70 patients with pHPT. Large C-PTH was calculated by iPTH minus whole PTH. Results are shown as the percentage of the higher value of either basal or pre-excision and means (s.e.). There was a significant difference in the percent decrease between them ($P < 0.001$) by a generalized linear regression analysis. Whole PTH was significantly lower than iPTH at all times by paired t -test.

Table 2 Comparisons of PTH levels among the bilateral internal jugular veins and the peripheral vein. Values are means $\pm$ s.d.

Measurements	Higher PTH side of the internal jugular vein	Lower PTH side of the internal jugular vein	Peripheral vein
iPTH	393 $\pm$ 441 (55–1520) ^a	121 $\pm$ 85 (42–468)	124 $\pm$ 97 (45–528)
Whole PTH	278 $\pm$ 303 (34–1110) ^a	78 $\pm$ 56 (18–286)	79 $\pm$ 60 (18–308)
Large C-PTH	117 $\pm$ 135 (22–510) ^b	43 $\pm$ 33 (13–182)	46 $\pm$ 40 (15–220)
% large C-PTH	32 $\pm$ 11 (4–52) ^b	37 $\pm$ 13 (18–69)	38 $\pm$ 12 (16–66)
Whole PTH/large C-PTH	2.59 $\pm$ 1.68 (0.93–8.32) ^b	2.01 $\pm$ 1.06 (0.44–4.42)	1.95 $\pm$ 1.15 (0.52–5.27)

iPTH, intact parathyroid hormone; Whole PTH, whole molecule parathyroid hormone; Large C-PTH, large carboxyterminal parathyroid hormone fragment.

*The large C-PTH was calculated by subtracting the whole PTH value from the iPTH value.

^a, $P < 0.001$; ^b, $P < 0.05$ (higher PTH side vs lower PTH side or peripheral).

$P < 0.001$). The plasma whole PTH and iPTH value of 70 patients dropped to 26 ± 10 and $32 \pm 12\%$ at 5 min, 15 ± 6 and $22 \pm 8\%$ at 10 min and 11 ± 5 and $17 \pm 7\%$ at 15 min from the higher value of either basal or pre-excision levels after removal of the enlarged gland respectively. At 5 min, the whole PTH assay levels of all 70 patients dropped to less than 50% of the pre-parathyroidectomy level; however, five (7%) patients retained their iPTH assay levels above 50% of the pre-parathyroidectomy level. The whole PTH/large C-PTH ratio decreased significantly after excision of one enlarged parathyroid gland (Fig. 3). The plasma large C-PTH value decreased slowly with a large variation, i.e. $60 \pm 53\%$ at 5 min, $51 \pm 53\%$ at 10 min and $45 \pm 61\%$ at 15 min. The half-life (means $\pm$ s.e.m.) of whole PTH was significantly shorter than that of iPTH or large C-PTH (2.33 ± 0.09 vs 2.82 ± 0.13 , or 9.89 ± 3.30 min, $P < 0.001$).

Discussion

In the present study we were able to demonstrate that the large C-PTH, likely 7-84 PTH, is directly secreted

from the hyper-functional parathyroid gland of adenomas in humans with pHPT. The principle of this study was based on the well-known fact that the parathyroid adenoma secretes abnormally high level of biologically active PTH that lead to hypercalcemia. On the other hand, the abnormally high levels of extracellular ionized calcium suppress the synthesis and release of PTH into the bloodstream in other normal parathyroid glands. Therefore, the difference between the adenoma-side internal jugular sample and the contralateral side is useful clinically to localize the hyper-functioning adenoma before (19) or during operation (20, 21). In this study, samples from the bi-lateral internal jugular as well as peripheral samples were measured for iPTH, whole PTH and the large C-PTH levels. It was observed that the mean levels of large C-PTH of the adenoma side were at least three times higher ($P < 0.001$) than those of the contralateral side and the peripheral vein, while there was no significant difference in large C-PTH levels between the non-adenoma side and the peripheral vein ($P = 0.184$). This clearly indicated that the adenoma produces and releases the large C-PTH directly; this finding is in agreement with earlier findings demonstrating intra-glandular production of the large C-PTH by the hyper-functional human parathyroid gland of secondary HPT patients with end-stage renal disease (9), as well as pHPT (22).

A great advance in our knowledge of the parathyroids was recently achieved with the discovery of the biological activity of 7-84 PTH. Slatopolsky *et al.* (9) were able to demonstrate that not only can the 7-84 PTH neutralize the hypercalcemic effect of 1-84 PTH but 7-84 PTH can also produce a significant decrease in plasma calcium in PTX rats. Nguyen-Yamamoto *et al.* (23) confirmed this finding in a significant study which further demonstrated that the 7-84 PTH alone exerts a hypophosphatemic effect by acting possibly through a C-PTH receptor, especially in the presence of 1-84 PTH. Divieti *et al.* (24) further demonstrated that the 7-84 PTH reduced osteoclast formation and reduced bone resorptive activity *in vitro*. Faugere *et al.* (16) reported that 7-84 PTH not only antagonized the hypercalcemic effects of 1-84 PTH, but also antagonized the stimulatory effects of 1-84 PTH on bone

Figure 3 Decline of whole PTH/large C-PTH ratio after excision of one enlarged parathyroid gland in 70 patients with pHPT. Results are shown as means (s.d.). The whole PTH/large C-PTH ratio decreased significantly by paired *t*-test in all except from 10 min to 15 min after removal of the parathyroid.

turnover in TPTX rats. This finding has significant implications regarding the potential role of 7-84 PTH in the pathogenesis of a dynamic bone disease. It would be logical to expect to find that the circulating level of hypercalcemic 1-84 PTH would be higher than the level of hypocalcemic 7-84 PTH in the pHPT patient in order to explain the clinical hypercalcemia and high bone turnover of this patient group, although the relative biological potencies of 1-84 and 7-84 PTH in regulating bone turnover and calcium homeostasis have not been thoroughly elucidated. Our study reported here not only confirmed that the iPTH levels of the adenoma-side internal jugular vein were significantly higher than the contralateral side, but also demonstrated that the secretion of the hypercalcemic 1-84 PTH followed the same pattern as iPTH. Moreover, we also observed that a greater proportion of 1-84 PTH in comparison with large C-PTH (likely 7-84 PTH) was produced and released into the circulation in the adenoma-side internal jugular vein, as well as in the peripheral circulation with a greater than 1.0 ratio of 1-84 PTH to large C-PTH. These observations explain that it is not simply that the absolute value of iPTH increased in patients with pHPT, but that there was a significantly greater 1-84 PTH proportion (compared with large C-PTH) that is responsible for the development of hyperparathyroidism bone and hypercalcemia.

We found a difference between the right and left internal jugular iPTH assay levels only in about 50% of the patients studied. Furthermore, there were two cases in which the iPTH level measured at the internal jugular vein was lower at the adenoma side than that at the other side, resulting in two false negative cases. This may have been inevitable because our sampling procedure was not to select catheterization into veins in the neck and mediastinum. Taylor *et al.* (20) reported five wrongly assigned results from 23 patients examined by basically the same lateralization procedure as ours and four had adenomas of lower glands; this could possibly be explained by the fact that the main venous drainage of an inferior adenoma was to the innominate rather than to the internal jugular vein. It may have been caused by manipulation or pressure on the adenoma side of the neck before puncture of the internal jugular veins of the contralateral side (21).

Another interesting observation of this study was that the proportion of large C-PTH level to 1-84 PTH level was lower in the adenoma side of the internal jugular vein than in the contralateral side, as well as that of the peripheral vein ($P < 0.05$), while there was no difference between the normal side of the internal jugular vein and the peripheral vein. This phenomenon could be explained by the relatively shorter circulating half-life of 1-84 PTH than of large C-PTH. In other words, the relatively longer circulating half-life of large C-PTH contributed to a slight decrease of 1-84 PTH/large C-PTH ratio in the peripheral

circulation (Table 2). This observation suggested a longer half-life of large C-PTH than 1-84 PTH and this was clearly supported by the non-parallel decline of large C-PTH and 1-84 PTH during the first 5 min of peripheral plasma sampling (Fig. 2).

The QPTH assay is now the most useful adjunct to successful parathyroidectomy (18, 25–28). Our knowledge of the different circulating half-lives of large C-PTH, likely 7-84 PTH, from 1-84 PTH would be very helpful in designing a QPTH assay for the clinical monitoring of parathyroidectomy. Theoretically, we would choose to measure or monitor the PTH molecule, preferably 1-84 PTH, from the pool of circulating heterogeneous PTH fragments including the large C-PTH with the shortest half-life, so that the waiting period during the operation would be reduced and the cost of surgery would be lowered. Although the currently available QPTH assays are the second generation iPTH versions which detect both 1-84 PTH and large C-PTH, we strongly suggest that future PTH assays for this purpose should be third generation versions which measure 1-84 PTH exclusively, with no cross-reaction to large C-PTH.

In conclusion, we have demonstrated the production and release of large C-PTH directly from the hyperfunctional parathyroid gland of the adenoma in patients with pHPT. We have further found that the circulating half-life of large C-PTH is relatively longer than that of 1-84 PTH. It is suggested that an immunoassay detecting 1-84 PTH exclusively would be the best fit for intra-operative monitoring of parathyroidectomy by reducing the waiting period during surgery.

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