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Measurement of Parathyrin in Blood from Thyroid Veins: Two Radioimmunoassays Compared in Patients with Primary Hyperparathyroidism

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We measured parathyrin (PTH) in peripheral venous blood samples and in thyroid veins (both homolateral and contralateral to the lesion) in 13 patients with surgically confirmed parathyroid adenomas. Two different RIAs were used, one specific to the mid-region of the molecule (44-68, M-PTH), the other specific to the carboxy-terminal region (65-84, C-PTH). With the M-PTH assay we established a statistically significant multiple correlation ($P < 0.05$) between the PTH concentrations in blood from the peripheral and thyroid veins; no significant correlation was found when we used the C-PTH assay. Our results confirm the superiority of the M-PTH RIA over the C-PTH RIA for study of hormonal secretion in primary hyperparathyroidism.

In 1968, Berson and Yalow (1) observed that parathyrin (PTH) in the plasma of patients with primary hyperparathyroidism (HPT) is immunoheterogeneous, a finding subsequently confirmed and expanded by several laboratories (2, 3).

The immunoreactive PTH in plasma from hyperparathyroid patients is a complex mixture of the intact PTH (PTH 1-84) and various peptide sequences. There are two theories as to the origin of these circulating forms. According to the first, these forms are almost completely attributable to the peripheral metabolism of the secreted PTH (4, 5); the second considers that the PTH fragments could be released by the parathyroid gland under certain conditions, mainly as the result of an increased concentration of calcium (6, 7).

The availability of radioimmunoassays (RIAs) with antisera specific for different regions of the hormone has made necessary a complete knowledge of these phenomena for interpretation and use of the assays. Here, we used two types of RIAs, one of them carboxy-terminal specific (C-PTH) and the other mid-region specific (M-PTH), to determine PTH concentrations in thyroid venous blood for correlation with the concentrations measured in blood from peripheral veins.

Materials and Methods

Mid-region parathyrin (M-PTH). In one method (cat. no. 51L1; Immuno-Nuclear Corp., Stillwater, MN 55082), the

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antiserum used is specific for the 44-68 region of human PTH, with zero cross-reactivity with human hPTH sequences 1-34 or 65-84 (8). The standard is a PTH fragment, [Tyr⁴³]-hPTH⁴⁴⁻⁶⁸. Enzymatically prepared beef PTH (37-84) labeled with ¹²⁵I was used for the tracer. We separated free tracer from bound tracer with a precipitating complex containing normal chicken serum, rabbit or goat anti-chicken serum, and polyethylene glycol. The reference interval for PTH was 29 to 85 pmol/L. The limit of detection for the assays was 10 pmol/L. Samples from the same individual were always analyzed in duplicate in the same assay. The mean intra-assay CV was 4.5%.

Carboxy-terminal parathyrin (C-PTH). We also measured C-PTH with a method supplied by Immuno-Nuclear Corp. (cat. no. 1300). The antibody, developed against intact bovine PTH, reacts with the 65-84 sequence of human PTH (9). This RIA kit contains six standards of synthetic human PTH 65-84. The complex is precipitated by addition of a second antibody. The reference interval was from undetectable to 88 pmol/L. The minimum detectable concentration was 20 pmol/L, and the mean intra-assay CV was 5.0%.

Patients. We studied 13 surgically confirmed HPT patients with normal renal function, ages 40-65 y (mean 56, SD 9 y). In all cases the histological diagnosis was a single parathyroid adenoma. The concentrations of total calcium in serum ranged from 2.60 to 3.62 mmol/L (mean 2.87, SD 0.30 mmol/L).

After the patients had fasted overnight, we collected from each three blood samples from the cubital vein for basal (0800-1000 hours) pre-operative PTH (M-PTH and C-PTH) determinations. Samples were also taken during surgery, before removal of the adenoma, from the inferior thyroid veins, both homolateral and contralateral to the lesion, for use in determining PTH by both RIA methods.

Statistical analysis. After determining the PTH values in blood from peripheral and thyroid veins (homolateral and contralateral to the lesion), we performed a multiple linear correlation between these variables, considering each as a "dependent variable." In each case, we calculated the correlation coefficient (R^2), an absence-of-correlation test, and the corresponding regression equation. The results were considered significant at $\alpha = 0.05$ ($P < 0.05$). The confidence level (C) was calculated from the equation $C = (1 - P) \times 100$ (10).

Results

M-PTH. In the 13 cases studied, the mean (and SD) values for M-PTH (pmol/L) are shown in Table 1.

In the correlation study between M-PTH(x) and M-PTH(o) or M-PTH(p), with the PTH value for the peripheral vein as the dependent variable, there was a significant correlation ($P < 0.05$) with values for samples from the homolateral and contralateral thyroid veins: $R^2 = 0.5154$, $P = 0.0262$, regression equation: $M-PTH(p) = 0.12 \times [M-PTH(x)] + 0.18 \times [M-PTH(o)] + 8.76$.

Considering the PTH value for the sample from the thyroid vein homolateral to the lesion as the dependent variable, we observed a statistically significant correlation with the values for blood from the peripheral vein and from the thyroid vein contralateral to the lesion: $R^2 = 0.5606$, $P = 0.0162$, regression equation: $M-PTH(x) = 1.91 \times [M-PTH(p)] + 1.05 \times [M-PTH(o)] + 357$.

Likewise, with the PTH value for blood from the vein contralateral to the thyroid lesion as the dependent variable, we also found a statistically significant correlation with respect to the two other variables: $R^2 = 0.4752$, $P = 0.0417$, regression equation: $M-PTH(o) = 0.42 \times [M-PTH(p)] + 0.15 \times [M-PTH(x)] + 56.15$.

These results are summarized in Table 2.

C-PTH. The mean (and SD) values for C-PTH (pmol/L) were: peripheral vein (p) = 98 ± 83 , homolateral vein to the lesion (x) = 702 ± 243 , and the vein contralateral to the lesion (o) = 179 ± 152 (Table 1).

None of the correlation studies performed, considering each variable in turn as dependent, proved to be statistically significant (Table 2).

Figure 1 shows the confidence level for the multiple

Table 1. Concentrations M-PTH and C-PTH in Peripheral and Thyroid-Vein Blood Samples from 13 Patients

	M-PTH(p)	M-PTH(x)	M-PTH(o)	C-PTH(p)	C-PTH(x)	C-PTH(o)
	Concn, pmol/L					
	100	1500	840	47	700	520
	620	3300	720	320	600	310
	90	1050	310	87	500	275
	194	1300	141	62	720	60
	148	682	154	86	720	74
	157	785	357	92	1000	237
	101	761	154	72	520	120
	96	690	130	71	1000	83
	78	750	96	54	440	49
	540	994	611	230	1000	380
	89	724	74	41	1000	51
	142	798	139	74	690	68
	220	492	296	36	260	98
Mean	198	1063	309	98	702	179
SD	175	725	255	83	243	152

(p): peripheral vein; (x): thyroid-vein homolateral to the lesion; (o): thyroid-vein contralateral to the lesion.

Table 2. Multiple Linear Correlation between Values for Blood from the Peripheral and Thyroid Veins

Variables	Correlation, R^2	P
M-PTH(x), M-PTH(o)/M-PTH(p)	0.5154	0.0267
M-PTH(p), M-PTH(o)/M-PTH(x)	0.5616	0.0162
M-PTH(p), M-PTH(x)/M-PTH(o)	0.4702	0.0417
C-PTH(x), C-PTH(o)/C-PTH(p)	0.2074	0.3120
C-PTH(p), C-PTH(o)/C-PTH(x)	0.0259	0.8770
C-PTH(p), C-PTH(x)/C-PTH(o)	0.2029	0.3218

p, x, o, as in Table 1.

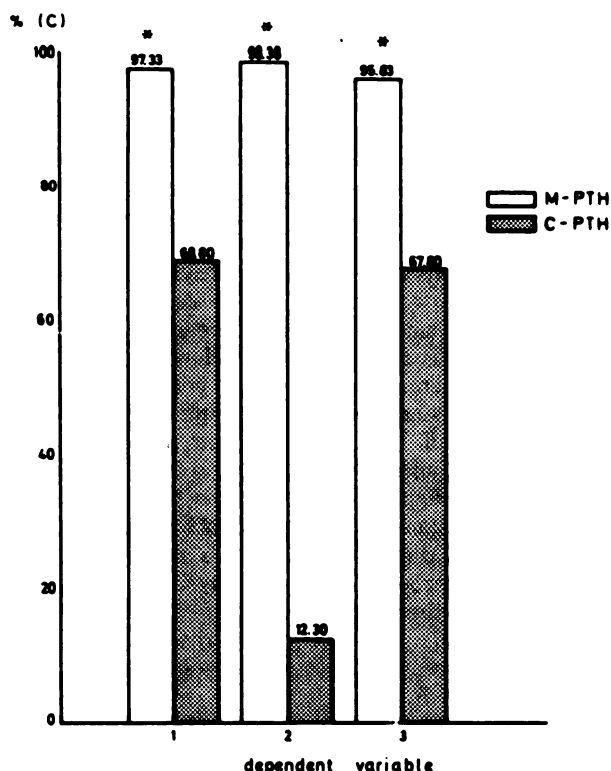


Fig. 1. Confidence level (C) for the multiple correlation analysis of the correlation between the PTH values for blood from the thyroid and peripheral veins, as determined with the M-PTH and C-PTH RIAs (1) peripheral vein; (2) thyroid vein homolateral to the lesion; (3) thyroid vein contralateral to the lesion; *, differences between M-PTH and C-PTH measurements were significant ($P < 0.05$) at each sampling site

correlation analysis between values for PTH in blood from the thyroid and peripheral veins as measured with the M-PTH and C-PTH RIAs.

Discussion

PTH determination is considered to be of primary importance in the study of mineral metabolism, and essential for the diagnosis of HPT (11). Nevertheless, determination and interpretation of the results of radioimmunoassayable PTH are rather complex, mainly owing to the heterogeneity of the circulating forms (12). In this sense, the current availability of PTH assays with different regional specificity has added to the debate concerning their true diagnostic usefulness (13).

It has been shown that the carboxy-terminal assays are superior to amino-terminal assays for diagnosis of chronic PTH hypersecretion, in spite of the absence of biological activity in this fragment (14, 15). However, there is a controversy over the theoretical basis and benefit of the mid-region-specific PTH assays (16), so that excellent results have been obtained by some using this method (17, 18) and not so satisfactory results by other investigators, who urge the use of "intact molecule" RIAs (19, 20).

Roos et al. (21), using a mid-region-specific RIA for serum and glands of HPT patients, suggest that there is a production of "M-fragments" in adenomatous glands. Similar findings were reported by Marx et al. (22) and Mallette et al. (23), using complex gel-filtration studies. However, Kubler et al. (24), investigating PTH secretion in cell cultures of parathyroid adenomas, were unable to demonstrate the presence of M-fragments and only a very small proportion of

carboxy-terminal residues.

Our results shed new light on the evaluation of the hormonal secretion of HPT patients. In our study, the mean values for M-PTH in blood from the thyroid vein homolateral to the lesion were threefold those for the contralateral side, and a significant correlation between M-PTH values for blood from the peripheral and thyroid veins (homolateral and contralateral to the lesion) was found irrespective of which was designated the dependent variable. The mean C-PTH concentrations in blood from the thyroid vein homolateral to the lesion were also about threefold those found in the contralateral side, suggesting increased production or secretion of C-PTH from the tumor. However, the lack of correlation between concentrations in peripheral and thyroid veins clearly suggests that the peripheral values do not reflect the rate of hormonal secretion in patients with parathyroid adenomas.

Our findings support the hypothesis that suggests an increased production of these fragments in HPT, and the results also show the superiority of the mid-region-specific assay over the carboxy-terminal assay in these patients. Nevertheless, further investigations are necessary to confirm these findings and also to contrast them with the intact-molecule assays.

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