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Circulating forms of parathyroid hormone detected with an immunofluorometric assay in patients with primary hyperparathyroidism and in hyperparathyroidism secondary to chronic renal failure

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Abstract

In patients with uremia, intact parathyroid hormone (PTH) measurement appears to overestimate the biologically active hormone in circulation. The recent description of the accumulation in these patients of a non-intact PTH form measured by the standard immunometric assays, re-opened the question. In this study we submitted serum samples from 7 patients with primary hyperparathyroidism (PHP) and from 10 patients with hyperparathyroidism secondary to chronic renal failure (SHP) to preparative HPLC in order to discriminate the molecular forms measured by our currently used immunofluorometric assay for intact PTH. The elution profile obtained with the HPLC system showed two clearly defined peaks, the first one corresponding to a lower molecular weight form, and the second to the intact PTH (1-84) form. In patients with SHP the area under the curve for the first peak (mean 29.5%, range 20.6 to 40.4%) was significantly greater than that observed for patients with PHP (mean 15.6%, range 5.6 to 21.9%). This confirms previous studies showing accumulation of molecular forms of slightly lower molecular weight, presumably PTH (7-84), in patients with SHP and, to a lesser extent, in patients with PHP. The real necessity of assays that discriminate between these two molecular forms is debatable.

Key words
- Parathyroid hormone
- Parathyroid hormone fragment
- Immunometric assay
- Primary hyperparathyroidism
- Hyperparathyroidism secondary to chronic renal failure

Parathyroid hormone (PTH) is a linear peptide of 84 amino acids produced exclusively by the parathyroid glands whose main function is to regulate calcium levels in the body. PTH circulates at very low concentrations, and its measurement has evolved substantially since the first description of a radioimmunoassay that was capable of measuring the concentration of this hormone in normal and pathological conditions (1). The early assays had carboxyl-terminal specificity, being subsequently supplanted by amino-terminal specific assays that presented better clinical utility, especially in patients with some degree of renal insufficiency (2). This is due to the fact that the metabolically inac-

tive carboxyl-terminal fragments accumulate as glomerular filtration rates decline, and under these conditions an assay specific for the biologically active amino-terminal segment is more appropriate (3). These methodological discussions seemed to have ended with the description of the first immunometric assay for PTH (4), that, recognizing the hormone via two antibodies, one specific for the carboxyl-terminal and other for the amino-terminal sequence, could only measure the intact hormone molecule. The high sensitivity of these assays, associated with their remarkable specificity, provided a powerful new tool for the diagnosis of PTH-related diseases (5).

PTH structure and function studies have shown some very interesting characteristics of the molecule: one of them is that the biological activity is concentrated in the amino-terminal section of the molecule. In fact, deletion of the first two amino acids leads to a peptide that binds to the receptor but is almost devoid of biological activity (6). Studies based on hydrophilicity plots and on the characteristics of most antibodies produced against the amino-terminal segment of the molecule showed that the dominant epitope of this section of the PTH molecule is located in the region between amino acids 15 and 26 (7). This information, together with the clinical observation that in patients with uremia intact PTH measurements appeared to overestimate the biologically active hormone in circulation (8), indicated that perhaps in these patients some biologically inactive form was being measured by the “intact” hormone assays. In fact, a loss of a small number of amino-terminal amino acids will produce a biologically inactive PTH form that theoretically will still be recognized by the current intact assay systems. Brossard et al. (9) were the first to demonstrate that in patients with chronic renal failure there was an accumulation of a non-(1-84) PTH form measured by the immunometric assays. The importance of this finding is evident, and the view that the current immunometric assays for the intact molecule were in fact measuring only the biologically active PTH (1-84) form turned out to be questionable.

In this study we submitted serum samples from patients with primary hyperparathyroidism (PHP) and from patients with hyperparathyroidism secondary to chronic renal failure (SHP) to high-performance liquid chromatography (HPLC) in order to discriminate amongst molecular forms measured by our currently used immunofluorometric assay for intact PTH (5).

Serum samples from 7 patients with surgically proven PHP and from 10 uremic patients under ongoing dialysis and with SHP were submitted to the HPLC procedure. The patients were treated at the Endocrinology and Nephrology Clinics of Escola Paulista de Medicina, Universidade Federal de São Paulo. The PTH levels from the PHP patients ranged from 76 to 1837 pg/ml (median 219 pg/ml) and the levels from the patients with SHP ranged from 1036 to 1717 pg/ml (median 1324 pg/ml). Prior to the chromatographic separation, serum samples were pre-purified on Sep-Pak C_{18} cartridges, and the adsorbed PTH was eluted and evaporated under nitrogen. Samples were then reconstituted in 1 ml of a solution of 0.1% trifluoroacetic acid (TFA) in water. The chromatographic system used was based on that described by Brossard et al. (9). Fifty microliters was then injected into a Sephasil Peptide C_{18} analytical column (4.6 x 250 mm; Pharmacia Biotech, Uppsala, Sweden) and eluted using a noncontinuous linear gradient of acetonitrile (0-50% in 0.1% TFA in water, applied at time = 10 min) run for 60 min in a Waters HPLC system. One-milliliter fractions were collected, and the selected tubes were evaporated under nitrogen and reconstituted with 1 ml of PTH assay buffer. The immunofluorometric assay employed (5) recognizes PTH via two epitopes: one carboxyl terminal located around amino acids 68-69,

and one amino terminal composed of amino acids 15-26 (7). The area under the curve was calculated using the Inplot software (GraphPad Software, San Diego, CA, USA).

The elution profiles obtained by HPLC for serum samples from one patient with PHP and one with SHP are shown in Figure 1. Two elution peaks are clearly visible: a first peak (tubes 41-43) and a larger second peak (tubes 45-47). According to Lepage et al. (10), the first peak corresponds to PTH (7-84) and the second to intact PTH (1-84). Calculation of the area under the curve corresponding to the first peak for the 7 samples from patients with PHP showed a mean of 15.6%, with a minimum of 5.6% and a maximum of 21.9%; for the patients with SHP the mean area under the curve for the first peak was 29.5%, with a minimum of 20.6% and a maximum of 40.4% (Figure 2). Statistical analysis showed that this difference was significant (P = 0.0002, Mann-Whitney test).

Our findings agree with those described by Brossard et al. (9) and Lepage et al. (10) in that patients with SHP have a significant proportion of hormone measured by intact PTH assays that are not 1-84 molecules. This finding could explain the lack of correlation between the severity of bone abnormalities and intact PTH levels observed in patients with chronic renal failure (8). One of the possible reasons for the finding of PTH levels higher than expected in these patients could be the presence of a molecular form recognized by the routine intact assays but lacking some amino-terminal amino acids. Considering the specificity characteristics of most of the assays in use, this finding is not surprising, and the questions are: what kind of PTH form is measured, when it appears, and what is the clinical importance of these new molecular forms. Data have been published showing that a correlation exists between the decrease of glomerular filtration rate and the non-(1-84) PTH forms (11). Nonetheless, the finding that these truncated forms are present in patients with primary hyperparathyroidism and also in normal individuals (12), albeit at lower concentration, indicates that the phenomenon is not exclusive of patients with chronic renal failure.

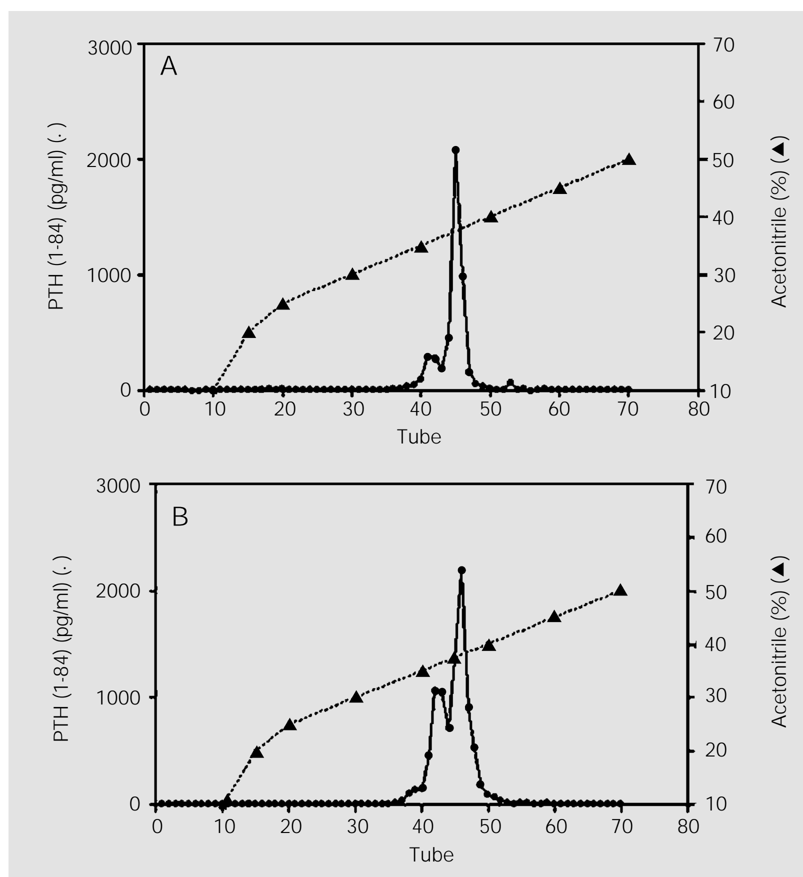

Figure 1. Elution profiles obtained by HPLC for serum from a patient with primary hyperparathyroidism (A) and from a patient with hyperparathyroidism secondary to chronic renal failure (B). PTH (1-84) = parathyroid hormone 1-84.

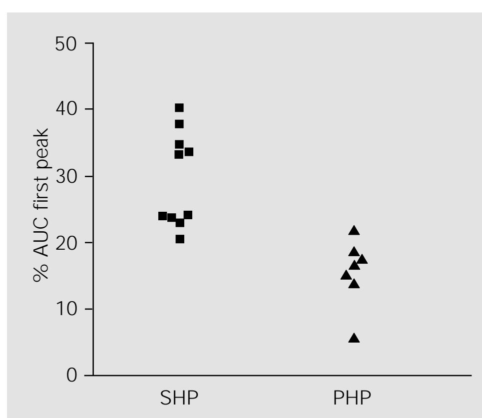

Figure 2. Percent area under the curve (AUC) corresponding to the first chromatographic peak for the 7 patients with primary hyperparathyroidism (PHP) and for the 10 patients with hyperparathyroidism secondary to chronic renal failure (SHP).

The recent description and characterization of an assay exclusively specific for the active whole PTH (1-84) form (13) confirmed values lower than those obtained by the traditional "intact" PTH assays (like ours) in patients with hyperparathyroidism and in normal individuals. The discrimination between normal and hyperparathyroid patients does not appear to be significantly better than that obtained with the "intact" assays, but more data are necessary for this conclusion. The recent description that the PTH (7-84) fragment can have hypocalcemic actions via a receptor different from the PTH/PTH-related peptide receptor indicates that this form is not simply a carboxyl-terminal fragment (14). This finding is a strong argument in favor of the necessity of measuring both forms, at least in patients with chronic renal deficiency and SHP, in order to have a more complete evaluation of parathyroid function.

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