Parathyrin Radioimmunoassay: Diagnostic Utility of Antisera Produced against Carboxyl-terminal Fragments of the Hormone from the Human

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Antisera directed toward the carboxyl-terminal region of human parathyrin (parathyroid hormone), for use in diagnostically applicable radioimmunoassays of the hormone in serum, are scarce, largely because of the lack of suitable immunogens of human origin. We produced four antisera in goats and guinea pigs by immunization with recently discovered carboxyl-terminal fragments of human parathyrin extracted from parathyroid tumors. Here, we report results of radioimmunoassays of nearly 200 normal and pathological sera with one of these antisera; we observed almost complete differentiation between concentrations of parathyrin in serum of healthy normal subjects and patients with primary, secondary (due to chronic renal failure), or “ectopic” hyperparathyroidism (due to non-parathyroid cancer). The availability of a new immunogen should now make possible the deliberate production of large quantities of diagnostically applicable parathyrin antisera directed toward the carboxyl-terminal region of human parathyrin. This should, in turn, lead to more widespread availability of this useful radioimmunoassay.

Measurement of parathyrin (parathyroid hormone) in human serum by radioimmunoassay has widely-recognized clinical applicability in diagnosis of parathyroid gland dysfunction (1–4). Unfortunately, it has not yet attained the status of a universally available diagnostic test; few laboratories possess the necessary reagents to perform it satisfactorily on a routine basis.

Several factors have prevented more widespread adoption of the parathyrin radioimmunoassay (5, 6). One of the most serious is a consequence of the immunoheterogeneity of parathyrin in blood: the hormone circulates in several molecular forms (7) and antisera used in its radioimmunoassay recognize some of these molecular forms but not others (8). Reports from several laboratories have now established that >80% of the immunoreactive parathyrin (iPTH) in the plasma of patients with primary hyperparathyroidism consists of biologically-inert, carboxyl (COOH)-terminal fragments of the hormone (9–13). Although their precise physiologic function remains obscure (8), these fragments have diagnostic importance: the key to a reliable radioimmunoassay for quantitating steady-state concentrations of iPTH for aid in the diagnosis of primary hyperparathyroidism appears to be its ability to detect these hormonal fragments (6, 11, 13).

There is also a critical shortage of satisfactory antisera for clinically applicable radioimmunoassays of serum iPTH. Because of the lack of suitable human parathyrin immunogens, antisera have had to be produced against preparations of bovine or porcine parathyrin (3, 4, 9, 10, 14); few such antisera have the high affinity and high degree of cross reactivity with the COOH-terminal region of human parathyrin needed to maximize diagnostic utility. Radioimmunoassays with many of these antisera also suffer from a high degree of overlap (as high as about 50%) between serum iPTH concentrations of normal subjects and patients with surgically proven primary hyperparathyroidism.

We recently reported that relatively large quantities of predominantly COOH-terminal fragments of human parathyrin can be extracted from pooled human parathyroid tumors (15). We also used this new immunogen to produce four antisera in guinea pigs and goats; all four of these antisera are directed toward the carboxyl-terminal region of human parathyrin (15). In the studies to be reported here, we have used a radioimmunoassay with one of these antisera to measure iPTH in nearly 200 serum specimens from both normal subjects and from patients with disorders of mineral homeostasis and parathyroid gland function. Our radioimmunoassay results with this new antisem—which readily detects COOH-terminal fragments of human parathyrin in serum (15)—show <5% overlap between serum iPTH concentrations in normal subjects and those with primary hyperparathyroidism. This is one of the lowest reported degrees of overlap of iPTH values between these two groups.

Materials and Methods

Production and characterization of antisera. The procedures used to produce antisera against COOH-terminal fragments of human parathyrin have been reported in detail elsewhere (15). In brief, these fragments are recovered from the trichloroacetic acid (75
g/liter) supernate, a by-product of the extraction of pooled human parathyroid tumors by the urea/tri-chloroacetic acid procedure (16). After concentration and desalting, the crude lyophilized parathyrin fragments (predominantly COOH-terminal) can be used as immunogens without further purification. Antiserum GPFM—that used in this work—was produced in a guinea pig immunized by the method of Vaitukaitis et al. (17). This antiserum has been fully characterized (15); it recognizes the 53–84 amino acid sequence region of the bovine parathyrin molecule. Recognition for other regions of the parathyrin molecule could not be detected at the dilution used (10,000-fold final dilution).

Parathyrin assay standards. Bovine and human parathyrin were extracted from parathyroid glands or parathyroid tumor tissue, respectively, and purified to homogeneity by published methods (18).

Radioimmunoassays. The procedure of Arnaud et al. (14) was used with only minor modifications. Assays were done under nonequilibrium conditions at 4 °C, with shaking; the assay diluent consisted of sodium barbital buffer (0.1 mol/liter, pH 8.6) containing 10% by volume hypoparathyroid plasma and aprotinin (Trasylol; FBA Pharmaceutical Co.), 500 kIU/ml. Tubes were incubated with antiserum GPFM (final dilution, 10,000-fold) for three days, followed by incubation with 5000 cpm of 131-I-labeled bovine parathyrin (19) for two additional days. Antibody-bound was separated from “free” tracer with dextran-coated charcoal by a modification of the procedure of Herbert et al. (14, 20). Both “bound” and “free” tracers were counted for 131-I (Searle Analytic Instruments, Des Plaines, Ill. 60018) after centrifugation and separation of supernates (“bound”) and precipitates (“free”). Assay results were calculated by previously published procedures (14), and values for serum specimens were read directly from the assay standard curves.

Serum specimens. Sera were collected from 40 healthy normal subjects at 0700–0800 hours, after overnight fast. These individuals, 19 men and 21 women, ranged in age from 18 to 57 years. Sera were also obtained from the following groups of Mayo Clinic patients: proved primary hyperparathyroidism (64 patients)—these individuals all had a parathyroid adenoma or hyperplastic parathyroid glands subsequently removed at surgery; suspected primary hyperparathyroidism (81 patients)—these patients had renal, osseous, or other manifestations of the disease, but no surgical confirmation; secondary hyperparathyroidism (12 patients)—these individuals all had end-stage chronic renal failure (endogenous creatinine clearance, <10 ml/min per 24 h); ectopic hyperparathyroidism (15 patients)—this group consisted of individuals who were hypercalcemic as a result of nonparathyroid cancer, with or without skeletal metastases; hypoparathyroidism (12 patients)—these individuals had had total surgical removal of all parathyroid tissue.

All serum samples were no more than six months old and were assayed at three or more dilutions. Reported values are the arithmetic means for at least three of these dilutions.

Parathyrin was considered to be undetectable in the serum when duplicate 200-μl samples failed to produce depression of the bound/free (B/F) ratio greater than that observed with 200-μl samples of serum from surgically hypoparathyroid patients (i.e., ≤10% depression).

Serum calcium. Serum calcium was measured by atomic absorption spectroscopy (21). The normal range for serum calcium at our institution is 89–101 mg/liter.

Miscellaneous. Statistical methods were those of Dixon and Massey (22).

Results

Standard curves. Figure 1 shows typical radioimmunoassay curves with pure human parathyrin or pure bovine parathyrin as the reference standards. The curves with the two species of parathyrin were parallel from about 15 to 85% of “trace” (initial B/F ratio). In the seven assays in which both reference standards were run simultaneously, the ratio pg human parathyrin/pg bovine parathyrin was 1.99 ± 0.25 (SD) at 80% of “trace”, 1.94 ± 0.28 at 50% “trace,” and 1.73 ± 0.11 at 20%. None of the differences between these ratios is statistically significant (P > 0.05).

The immunodilution curves of these parathyrin reference standards and those of all serum specimens assayed were parallel. Therefore, assay results for parathyrin could validly be expressed in terms of either of these two reference standards. In the discussion that follows, all values for parathyrin are expressed as picogram equivalents (pg eq) of human parathyrin per milliliter. The detection limit in this radioimmunoassay was about 60 pg of pure human parathyrin per tube; this corresponds to a sensitivity of about 300 pg eq/ml, with use of the maximum possible serum sample volume (200 μl).

Serum parathyrin in normal subjects and patients. Figure 2 summarizes serum iPTH values in normal subjects and in various patient groups. Serum iPTH was
undetectable (<300 pg eq of human parathyrin per milliliter) in two of the 40 normal subjects (44 specimens), in one of 15 patients with “ectopic” hyperparathyroidism, and in all 12 of the hypoparathyroid sera examined. In the normal group the iPTH values were normally distributed, and the mean (±SD) was 560 ± 111 pg eq of human parathyrin per milliliter; the mean ±2 SD range was 339–781. Of 73 serum specimens from the patients with surgically proved primary hyperparathyroidism, only three (4%) had iPTH values that fell in this range; in this group, iPTH ranged from 610 to 48,700 pg eq of human parathyrin per milliliter. In the 19 patients suspected of having primary hyperparathyroidism, iPTH ranged from 490–1810 pg eq of human parathyrin per milliliter; four (21%) had values within the range of normal (mean ±2 SD). The patients with secondary hyperparathyroidism due to chronic renal failure had iPTH concentrations ranging from 3300 to 76,500 pg eq of human parathyrin per milliliter; the patients with “ectopic” hyperparathyroidism had normal or slightly increased iPTH concentrations (range, undetectable to 950 pg eq/ml).

A plot of the serum iPTH values vs. serum calcium (Figure 3) revealed no statistically significant correlation between these two variables in normal subjects. In the proven primary hyperparathyroid group, however, we observed a highly significant positive correlation (r = .52, P < .001) between serum iPTH and serum calcium. The three patients in this group who had iPTH concentrations in the range of normals (±2 SD) were clearly differentiated from the normal subjects in this type of plot. The patients with “ectopic” hyperparathyroidism were readily differentiated from normal subjects and almost completely differentiated from the patients with proven primary hyperparathyroidism.

Precision. For 33 measurements of iPTH (made on 20 separate days) in a pooled specimen of serum from primary hyperparathyroid patients, the mean (±SD) was 6640 ± 512 pg eq of human parathyrin per milliliter, with a CV of 7.7%. For 20 consecutive iPTH values (taken on the same day) of a low and high serum pool, CV’s were 5.3 and 3.3%, respectively. In analytical recovery studies, recovery of iPTH ranged from 85 to 107% (mean, 96.7%) (Table 1), and the correlation coefficient (picograms of pure human parathyrin added to hypoparathyroid serum vs. pg parathyrin measured) was 0.98.

**Discussion**

The parathyrin radioimmunoassay described in this paper offers three advantages over earlier ones.

First, with the exception of the assay described by Fischer et al. (13), our antisera is the only one reported that has been produced solely against a preparation of human parathyroid extracts. Radioimmunoassays with guinea pig antisera GPFM thus overcome the potentially critical problem of cross reactivity: whether or not antisera produced against nonhuman parathyroid hormones are capable of detecting and adequately quantitating human parathyrin or its fragments in serum. Although we have no conclusive chemical evidence that the peptide fragments against which this antisera was produced and the iPTH fragments in hyperparathyroid serum are identical, available information strongly suggests that they are (15). Therefore, it is logical to expect that antisera GPFM (and other parathyrin antisera produced against

**Table 1. Analytical Recovery of Pure Human Parathyrin Added to Hypoparathyroid Serum**

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<tr>
<th>Added (pg/200-μl sample)</th>
<th>Measured (mean ± SD; n = 3)</th>
<th>Mean recovery %</th>
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<tr>
<td>200</td>
<td>199 ± 12</td>
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<td>150</td>
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<td>80</td>
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similar immunogens) would recognize the principal iPTH species in hyperparathyroid serum well.

Secondly, radioimmunoassays with GPFM give one of the lowest percentages of overlap of any published parathyrin radioimmunoassay between values for normal subjects and patients with proven primary hyperparathyroidism (3, 4, 8, 13, 14). To be of maximum clinical diagnostic utility, a radioimmunoassay for parathyrin must clearly discriminate between these two groups. Because antiserum GPFM has been produced against major molecular species of iPTH in the serum of hyperparathyroid patients, its diagnostic utility might be expected. The small (<5%) overlap between normal and pathological iPTH values can be essentially completely obliterated by formal discriminant analysis, plotting serum calcium vs. serum iPTH concentration (6) (Figure 3). This radioimmunoassay also shows excellent ability to discriminate normal sera from those of patients with secondary hyperparathyroidism.

Thirdly, many antisera to parathyrin previously described show reactions of immunologic nonidentity between "glandular and "secreted" parathyrin peptides (6–11). This fact has precluded the use of the pure hormone as a radioimmunoassay standard and has frequently made it necessary for each laboratory performing such radioimmunoassays to express iPTH values in units of its own arbitrarily-selected hyperparathyroid serum (6, 8). This practice has, in turn, often made it difficult to compare iPTH results directly from one radioimmunoassay to another. The inability of antiserum GPFM to distinguish immunologically between parathyrin extracted from parathyroid glands and iPTH species in hyperparathyroid serum makes possible the desirable feature of expressing radioimmunoassay results with this antiserum in gravimetric units with pure parathyrin as assay standard. Results can be more easily compared between laboratories when these more universal units are used.

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References


