Radioimmunoassay for Somatomedin C: Comparison with Radioreceptor Assay in Patients with Growth-Hormone Disorders, Hypothyroidism, and Renal Failure

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We raised an antiserum (Tr4) in rabbits against a basic somatomedin C-like peptide preparation. Using high-immunoreactivity somatomedin C tracer, we compared the performance of radioimmunoassays in which we used the Tr4 antiserum and a well-characterized somatomedin C antiserum distributed by the National Pituitary Agency (NPA) with that of the human placental-membrane somatomedin radioreceptor assay (RRA). In their cross reactivity towards various somatomedin-like and unrelated peptides, the two radioimmunoassay methods were almost identical, although NPA antiserum, with about fourfold higher titer than Tr4 antiserum, showed a slightly greater sensitivity for most peptides tested. Radioimmunoassay of acid-ethanol-extracted plasma samples from normal persons and acromegalic, hypopituitary, hypothyroid, and renal-failure patients revealed no analytical differences between the antisera (for 122 samples, r = 0.979 between methods). Somatomedin values for acromegalic and hypopituitary samples showed no overlap with normals. Values for hypothyroid and pre-dialysis renal-failure samples were significantly lower than normal. By comparison, the RRA showed greater cross reactivity towards some somatomedin-like peptides and gave significantly lower values than radioimmunoassay for acromegalic and hypothyroid plasma extracts, and significantly higher values for hypopituitary and renal-failure samples. We conclude that the radioimmunoassay methods clearly are of greater diagnostic value than RRA for clinical somatomedin measurement.

Additional Keyphrases: somatomedin • status of the patient • insulin-like growth factors • acromegaly • hypothyroidism • hypopituitarism • renal failure • cross reactivity • reference interval • effect of hemodialysis • dependence on thyroid hormone status

The somatomedins, a group of growth-hormone (somatotropin)-dependent anabolic and mitogenic peptides, include somatomedin A, somatomedin C, the insulin-like growth factors IGF-1 and IGF-2 (formerly known as "nonsuppressible insulin-like activity"), and multiplication-stimulating activity (MSA) (1). Of these, only IGF-1 and IGF-2 have had full sequence data reported, and they have been shown to be homologous with respect to 62% of their amino acids (2). Although somatomedin C has been only partly sequenced, it appears to resemble IGF-1 in its N-terminal amino acids (3), and it is immunologically indistinguishable from IGF-1 by use of antibodies directed against the whole somatomedin C molecule (4), the "C-peptide" region of IGF-1 (5), or the C-terminus of IGF-1 (6). For this reason, the term "somatomedin C/IGF-1" has been proposed (4). Somatomedin A and MSA also cross react with somatomedin C antibodies (4, 7), and antiserum raised against somatomedin A actually shows greater reactivity towards IGF-1 than towards somatomedin A (8). Clearly there are many structural similarities between the somatomedins.

Measurement of somatomedin in serum is important clinically because it allows assessment of the growth-hormone status of a patient (1, 7-10) without the need for complicated and expensive dynamic testing (11). While somatomedin measurement has been possible for some years by bioassay (12, 13), radioreceptor assay (14-20), and competitive protein binding assay (21, 22), specific radioimmunoassays have been developed only in the past few years (2, 5, 7, 8, 23, 24). In the present report we describe the development of a radioimmunoassay for a basic somatomedin that is similar to somatomedin C/IGF-1, and compare its performance with that of a well-characterized specific somatomedin C radioimmunoassay and of a radioreceptor assay for somatomedin in which human placental membrane is used as the source of binding sites.

Materials and Methods

Materials

Peptides. MSA, epidermal growth factor, and fibroblast growth factor were obtained from Collaborative Research Inc., Waltham, MA 02154. Human somatomedin B was purchased from Kabi Diagnostica, Stockholm, Sweden. Ovine and human somatotropin and prolactin were provided by the National Pituitary Agency, Bethesda, MD 20014. Rat somatomedin, about 20 arb. units/mg, was generously donated by Dr. W. H. Daughaday, St. Louis, MO. Human and porcine insulin were from Novo Industri, Copenhagen, Denmark.

Procedures

Somatomedin preparation. The isolation of a basic (pI 8.6-8.8) somatomedin preparation by a modification of the somatomedin C isolation method of Svoboda et al. (3) is described elsewhere (25). This material has a specific activity of about 2000 arb. units/mg by somatomedin C radioimmunoassay, and stimulates sulfate incorporation into costal cartilage of fasted 16-day-old rabbits. In the insulin radioreceptor assay of Marshall et al. (14), 25% displacement of bound tracer insulin is obtained with 1 µg (per tube) of somatomedin or 2 ng of porcine insulin.

The radiiodination and purification of somatomedin is described elsewhere (25).

Somatomedin antisera. A well-characterized (7) specific

1 Nonstandard abbreviations: IGF-1 and IGF-2, insulin-like growth factors I and II; MSA, multiplication-stimulating activity; NPA RIA, assay with use of National Pituitary Agency antisemum; Tr4 RIA, assay with use of antiserum prepared as described here; and RRA, radioreceptor assay.

Received Aug. 25, 1981; accepted Dec. 7, 1981.

488 CLINICAL CHEMISTRY, Vol. 28, No. 3, 1982
antiserum to somatomedin C was obtained from the National Pituitary Agency. We used this antiserum as a reference against which to compare the antiserum raised in our laboratory as described below.

To prepare our antiserum, we made a conjugate of 1200 µg of our somatomedin preparation and 400 µg of ovalbumin (Sigma Chemical Co.; Cohn Fraction V) with glutaraldehyde (26). After dialysis against a 9 g/L solution of NaCl, about 640 µg of conjugate in 1 mL, emulsified with 1 mL of complete Freund's adjuvant, was injected at multiple sites into each of two 2-kg New Zealand White rabbits. These animals were bled at four and eight weeks, and boosted at eight weeks with 200 µg of conjugate in incomplete Freund's adjuvant. Blood drawn 10 days later from one rabbit contained antibody (designated "Tr4") suitable for radioimmunoassay.

**Extraction of plasma samples.** Heparinized plasma samples were stored at -20 °C until used. To release somatomedics from their binding proteins, we extracted all samples with acid–ethanol according to the method of Daughaday et al. (27), except that only 0.1 mL of each sample was extracted, other reagent volumes being adjusted proportionally. For centrifuging extracts we used a Beckman Microfuge B. Analytical recovery of somatomedin by this method is essentially complete (27). Extracts so prepared are diluted sevenfold as compared with the original plasma.

**Radioimmunoassays.** The assay with use of National Pituitary Agency antiserum is referred to throughout as "NPA RIA," that with use of antiserum Tr4 as "Tr4 RIA." Incubations and separations were performed as described elsewhere (25). We used antisera at 40 000-fold dilution for the NPA RIA and 10 000-fold for the Tr4 RIA, giving 25–30% binding of tracer in the absence of added sample or standard. The standard was an acid–ethanol extract, prepared identically to the extracts of patients' samples, of a normal human plasma pool (n = 6), assigned a potency of 1.0 arb. unit/mL. This was included in each assay at eight dose levels between 0.1 and 25 µL per tube. Acid–ethanol extracts of patients' samples were measured in duplicate at two dose levels, 50 µL and 100 µL of a 50-fold dilution in assay buffer. Standard curves were prepared by linear regression of logit-transformed data. Under these conditions the useful working range of both assays was 0.1 to 10 arb. unit/mL.

**Radioreceptor assay (RRA).** This assay involving the use of human placental membrane receptors was performed by our previously described modification (18) of the method of Marshall et al. (14), except that the tracer used (5000 cpm per tube) was the same as that used for radioimmunoassay. The placental-membrane preparation (150 µg of protein per tube) bound 30–35% of tracer in the absence of added sample or standard. The standard human plasma extract was run at eight dose levels between 0.25 and 50 µL per tube. We measured acid–ethanol extracts of patients' samples in duplicate at two dose levels, 50 µL and 100 µL of a 10-fold dilution in radioimmunoassay buffer. Under these conditions the useful working range was 0.1–5 arb. units/mL.

**Quality control.** Three plasma samples with somatomedin concentrations in the hypopituitary, normal, and acromegalic ranges were included in each assay for quality control, and were treated exactly like patients' specimens. Overall between-assay variances were calculated from 10 assays. To gain extra degrees of freedom for determination of within-assay errors, we ran extra replicate tubes of the control specimens in some assays. Data were combined by analysis of variance to eliminate between-assay effects, to give within-assay error estimates with 23 degrees of freedom at each dose level. We used an HP 9845 computer to construct precision profiles from within-assay error measurements, by the method of Baxter (29).

**Results**

**Specificity**

We evaluated the specificities of the three assay methods by measuring the displacement of bound somatomedin tracer by various related preparations (Figure 1). Of the peptides tested, the human somatomedin preparation (2000 arb. units/mg) was the most potent in all assays, causing 50% displacement at a mean concentration of 0.35 ng per tube (NPA RIA), 0.55 ng per tube (Tr4 RIA), or 6 ng per tube (RRA). In most cases, parallel curves were obtained with rat somatomedin, acid–ethanol extracted human plasma pool, and MSA, although rat somatomedin showed some deviation from parallelism at high concentration in the Tr4 RIA. Rat somato-

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**Fig. 1.** Displacement by various peptides of 125I-labeled somatomedin C from (top) NPA antiserum, 1:40 000 final dilution; (middle) Tr4 antiserum, 1:10 000; and (bottom) human placental microsomal membrane, 150 µg of protein. Peptides tested were basic somatomedin preparation, 2000 arb. units/mg (Sm); rat somatomedin (rSm), acid–ethanol extract of normal human plasma (hP), multiplication-stimulating activity (MSA), human insulin (hI), human and ovine growth hormone (GH) and prolactin (PRL), epidermal growth factor (EGF), fibroblast growth factor (FGF), and somatomedin B (SmB).
Table 1. Between- and Within-Assay Errors for Somatomedin in Quality-Control Specimens as Measured by Radioimmunoassay and RRA *

<table>
<thead>
<tr>
<th></th>
<th>NPA RIA</th>
<th>Tr4 RIA</th>
<th>RRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Between-assay (df = 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.23 ± 0.04 (18.4%)</td>
<td>0.21 ± 0.03 (13.8%)</td>
<td>0.32 ± 0.07 (21.7%)</td>
</tr>
<tr>
<td>Medium</td>
<td>1.18 ± 0.15 (12.7%)</td>
<td>1.21 ± 0.10 (8.7%)</td>
<td>1.20 ± 0.14 (11.5%)</td>
</tr>
<tr>
<td>High</td>
<td>5.51 ± 0.59 (10.7%)</td>
<td>5.72 ± 0.82 (14.3%)</td>
<td>3.79 ± 0.38 (10.0%)</td>
</tr>
<tr>
<td>B. Within-assay (df = 23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.22 ± 0.04 (19.7%)</td>
<td>0.20 ± 0.03 (13.3%)</td>
<td>0.36 ± 0.09 (24.8%)</td>
</tr>
<tr>
<td>Medium</td>
<td>1.17 ± 0.12 (9.9%)</td>
<td>1.06 ± 0.12 (11.8%)</td>
<td>1.46 ± 0.22 (14.8%)</td>
</tr>
<tr>
<td>High</td>
<td>5.78 ± 0.67 (11.5%)</td>
<td>6.29 ± 0.92 (14.6%)</td>
<td>4.45 ± 0.46 (10.4%)</td>
</tr>
</tbody>
</table>

* Values are expressed in arb. units/mL as means ± SD, with CV in parentheses. Between-assay errors are derived from means of duplicate determinations on control specimens assayed in 10 successive runs. Within-assay errors represent the error of a single measurement, and are pooled by analysis of variance from six assays in which both 2 and 12 replicates were run. To compare with between-assay errors, divide values by the square root of 2.

Somatomedin was more potent than MSA by RIA, but this order was reversed by RRA. The potency ratios for human somatomedin/rat somatomedin/MSA (measured at 50% displacement) were about 1/0.13/0.03 (for NPA RIA), 1/0.17/0.02 (for Tr4 RIA), and 1/0.05/0.5 (for RRA). Human insulin displaced in a non-parallel manner, but was about 25 000-fold less potent than the human somatomedin preparation with use of NPA antiserum, 50 000-fold less potent with use of Tr4 antiserum, and 250-fold less potent in the RRA. Human and ovine somatomedin, human and ovine prolactin, epidermal growth factor, fibroblast growth factor, and somatomedin B were without effect in the radioimmunoassays, while fibroblast growth factor at 1 μg per tube and epidermal growth factor at 2.5 μg per tube caused approximately 15% displacement of tracer in the RRA. IGF-1, IGF-2, and somatomedin A were not available for testing.

Quality Control

Measured over 10 runs for each method, the standard acid–ethanol extracted plasma pool (a sevenfold dilution of the original pool) caused 50% displacement of bound tracer somatomedin at 1.26 (SD 0.26) μL per tube for the NPA RIA, 2.20 (SD 0.52) μL per tube for the Tr4 RIA, and 17.4 (SD 2.5) μL per tube for the RRA, a reflection of the relative sensitivities of the three assays towards purified somatomedin.

Table 1A shows total between-assay variation for three quality-control specimens in the three assay methods. As observed with patients' specimens (see below), the hypopituitary (low) control consistently gave a higher value by RRA than by radioimmunoassay, while the reverse was true of the acromegalic (high) specimen. Although the between-assay CVs do not differ significantly among these methods, RRA is evidently the least-precise method in the hypopituitary range, while the Tr4 RIA is least precise for the acromegalic specimen.

Within-assay errors are shown in Table 1B. These errors represent the error in measurement for a single determination, as obtained by pooling data from several assays by using analysis of variance. The errors for means of duplicates are obtained by dividing by the square root of 2.

Clearly, within-assay errors account for most of the total between-assay variation in all assay methods. Because the acid–ethanol extraction was carried out only once for each specimen and thus could not contribute to within-assay errors, we conclude that the extraction of samples is not a major source of error, despite the technical difficulty of pipetting ethanol extracts.

Figure 2 shows precision profiles for the three assay methods, the data representing results for means of duplicates. Within-assay CVs exceed 10% at dose levels below approximately 1.5 arb. unit/mL for the RRA, 0.3 arb. unit/mL for the NPA RIA, and even lower for the Tr4 RIA.

Patients' Samples

Figure 3 shows results for plasma somatomedin measurements on 16 acromegalic and 13 hypopituitary patients referred to the Endocrinology Department at Royal Prince Alfred Hospital. Most acromegalics had undergone treatment (pituitary surgery, irradiation, or therapy with bromocryptine), but all cases were judged to be clinically active. The hypopituitary group excluded younger children, for whom normal somatomedin levels are reported (7) to be below the adult normal range (a result confirmed in our laboratory). All had no somatomedin responses to provocative stimuli. Values for 40 normal adult subjects in the three assays, expressed (in arb. units/mL) as mean ± SD, with the range of values in parentheses, were: 0.89 ± 0.24 (0.53–1.49) for the NPA RIA, 0.97 ± 0.28 (0.62–1.69) for the Tr4 RIA, and 0.93 ± 0.26 (0.50–1.48) for the RRA. By paired t-test, values obtained by Tr4 RIA significantly (p <0.001) exceeded those obtained by NPA RIA; in contrast, RRA values did not differ significantly from those by either radioimmunoassay.

Acromegalic patient samples gave significantly higher values (p <0.001) than normals in all assays. There was no overlap between acromegalic and normal ranges in any assay, although some treated, but clinically active, acromegalics had values as low as 2 arb. units/mL. By paired t-test, results for acromegalics were not significantly different in the two radioimmunoassays, while the RRA gave results approximately 20% lower than either radioimmunoassay (p <0.005 in both cases).

Hypopituitary patients' samples gave significantly (p <0.001) lower values than did samples from normal individuals in all assays. There was no overlap with normals in either radioimmunoassay. NPA RIA systematically gave 20% lower values for this group than did Tr4 RIA (p <0.01), although the magnitude of the difference is uncertain because the limit of sensitivity was taken as 0.1 arb. unit/mL and lower samples were assigned this value. By RRA, five of 13 samples read low-normal to normal, preventing any clear discrimination between hypopituitary and normal specimens. RRA values for hypopituitary patients averaged 2.5 times those obtained by NPA RIA (p <0.005) and twice those obtained by Tr4 RIA (p <0.005). Table 2A–C compares linear correlation coefficients among the three assay methods for normal, acromegalic, and hypopituitary samples.

Ten patients with primary hypothyroidism had somatomedin concentrations ranging from low to normal in the three assays (Table 3). Mean values were significantly lower than normal in all cases, about 30% lower by radioimmunoassay and
Fig. 3. Somatomedin concentration in plasma of normal (n = 40), acromegalic (n = 16) and hypopituitary (n = 13) adults, as measured by three different methods
For acromegalic (squares) and hypopituitary (circles) samples, each point represents a single specimen. Vertical lines represent the mean ± 1 SD for each group. Horizontal broken lines represent the upper and lower limits of the range of normal values.

55% lower by RRA. The slight difference between the two radioimmunoassays was significant by paired t-test (p < 0.05). The RRA on average gave lower values than those obtained by radioimmunoassay, 30% lower than NPA RIA, and 38% lower than Tr4 RIA. As shown in Table 2D, individual RRA values were not significantly correlated with those obtained by either radioimmunoassay.

Ten patients with chronic renal failure, who were undergoing regular hemodialysis in the Renal Unit, Royal Prince Alfred Hospital, were tested immediately before and after dialysis. Before dialysis, mean somatomedin concentrations were 20–25% below normal by radioimmunoassay (p < 0.01 by NPA RIA, p < 0.05 by Tr4 RIA) but not significantly different from normal by RRA. All assay methods showed a consistent increase in plasma somatomedin after dialysis (Figure 4). The increase was about 20% as measured by both radioimmunoassays (p < 0.05) and 30% by RRA (p < 0.05), so that values after dialysis were not different from normal by

Table 2. Comparison of Somatomedin Assay Methods: Linear Correlation Coefficients for Between-Methods Comparisons

<table>
<thead>
<tr>
<th>Plasma samples</th>
<th>n</th>
<th>NPA RIA vs Tr4 RIA</th>
<th>NPA RIA vs RRA</th>
<th>Tr4 RIA vs RRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Normal</td>
<td>40</td>
<td>0.954*</td>
<td>0.557*</td>
<td>0.603*</td>
</tr>
<tr>
<td>B. Acromegalic</td>
<td>16</td>
<td>0.942*</td>
<td>0.675*</td>
<td>0.910*</td>
</tr>
<tr>
<td>C. Hypopituitary</td>
<td>13</td>
<td>0.839*</td>
<td>0.891*</td>
<td>0.677*</td>
</tr>
<tr>
<td>D. Hypothyroid</td>
<td>10</td>
<td>0.944*</td>
<td>0.107*</td>
<td>0.223*</td>
</tr>
<tr>
<td>E. Renal failure</td>
<td>20</td>
<td>0.947*</td>
<td>0.674*</td>
<td>0.613*</td>
</tr>
</tbody>
</table>

The renal failure group comprised pre- and post-dialysis samples from each of 10 patients.

Significance of correlations by t-test: *p < 0.001; **p < 0.025; *NS: *p < 0.002; **p < 0.01.

Fig. 2. Computer-drawn (HP 9845) precision profiles for the NPA RIA (a), Tr4 RIA (b), and RRA (c).

Circles represent the within-assay SD of three quality control specimens run in repeated assays. The dotted line is the quadratic curve joining these three points. The solid line is the same curve plotted as CV instead of SD. "U" stands for "arbitrary units" in Figures 2–5.
Table 3. Plasma Somatomedin Concentrations in Primary Hypothyroidism, as Measured by Radioimmunoassay and RRA

<table>
<thead>
<tr>
<th></th>
<th>NPA RIA</th>
<th>Tr4 RIA</th>
<th>RRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (and SD), arb. units/mL</td>
<td>0.60 ± 0.23</td>
<td>0.68 ± 0.32</td>
<td>0.42 ± 0.18</td>
</tr>
<tr>
<td>Range, arb. units/mL</td>
<td>0.13 - 1.03</td>
<td>0.16 - 1.32</td>
<td>0.10 - 0.76</td>
</tr>
<tr>
<td>t-test vs normals</td>
<td>p &lt;0.001</td>
<td>p &lt;0.001</td>
<td>p &lt;0.001</td>
</tr>
<tr>
<td>t-test vs RRA (paired)</td>
<td>p &lt;0.05</td>
<td>p &lt;0.025</td>
<td>p &lt;0.05</td>
</tr>
</tbody>
</table>

The 10 patients were 22-64 years old. The mean plasma thyroxin concentration (± SD) was 28 ± 15 nmol/L (normal: 68-125), and all had above-normal plasma thyrotropin values.

Table 4. Comparison of Somatomedin Assay Methods: Linear Regression Analysis of 122 Patients' Samples *

<table>
<thead>
<tr>
<th></th>
<th>NPA RIA vs Tr4 RIA</th>
<th>NPA RIA vs RRA</th>
<th>Tr4 RIA vs RRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>0.959 ± 0.022</td>
<td>0.714 ± 0.026</td>
<td>0.722 ± 0.026</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.146 ± 0.039</td>
<td>0.350 ± 0.046</td>
<td>0.267 ± 0.048</td>
</tr>
<tr>
<td>Corr. coeff.</td>
<td>0.971 ± 0.005</td>
<td>0.929 ± 0.012</td>
<td>0.930 ± 0.012</td>
</tr>
</tbody>
</table>

A. Untransformed data

B. Log-transformed data

Slope            | 0.944 ± 0.018       | 0.686 ± 0.041 | 0.711 ± 0.043 |
| Intercept        | 0.042 ± 0.007       | 0.032 ± 0.017 | 0.001 ± 0.016 |
| Corr. coeff.     | 0.979 ± 0.004       | 0.836 ± 0.027 | 0.836 ± 0.027 |

* Values are ±SE. All correlations are significant (p <0.001).

radioimmunoassay, but significantly higher than normal (p <0.001) by RRA. In general, Tr4 RIA gave slightly higher values than NPA RIA; 17% higher before dialysis (p <0.025), 12% higher after dialysis (p <0.05). In contrast, a striking difference was seen between RRA and radioimmunoassay values in these patients. Compared with the NPA RIA, RRA values were 50% higher before dialysis (p <0.001) and 68% higher after dialysis (p <0.005). As compared with the Tr4 RIA, RRA values were 35% higher before dialysis (p <0.01), 50% higher after (p <0.02). Table 2E compares correlation coefficients between the three assay methods for renal failure samples (pre- and post-dialysis results combined).

Finally, linear regression analysis was performed on 122 patients' samples (including the groups discussed above) measured in the three different assays. Table 4A summarizes the results for comparisons of the three assays. For the comparison between radioimmunoassay methods, the slope is 0.959 and the intercept 0.146. While all correlation coefficients are high, the slopes for regression lines where the RRA is treated as the dependent variable are significantly less than one.

Because values for most specimens fall in the range 0-2 arb. units/mL, linear regression on raw data becomes biased by the relatively few acromegalic samples. Therefore we repeated the analysis on log-transformed data to give more equal weighting to values across the analytical range. Table 4B shows that, while the correlation coefficient for the comparison of radioimmunoassays increased slightly to 0.978, values for the RRA-radioimmunoassay comparisons both fell to 0.836, reflecting poorer correlations between these assay methods in the normal and low ranges.

Relationships between results obtained in the three assays can be seen clearly in Figure 5, in which log-transformed data are plotted.

Discussion

In the past few years there has been a growing awareness of the importance of somatomedin measurement in the diagnosis and treatment of growth-hormone-related disorders. This has led to the development of radioimmunoassays in several laboratories, but none currently is available as a
commercial kit, although one highly specific, well-characterized antiserum is distributed by the National Pituitary Agency in limited quantities.

Here we have described our evaluation of a somatomedin antiserum generated in our laboratory. The evaluation is based both on the performance of the antiserum towards plasma samples from patients with acromegaly, hypopituitarism, hypothyroidism, and renal failure, and on a comparison of the performance of this antiserum with that of the well-characterized antiserum described above. Because a human placental preparation is much easier to prepare than an antiserum, we have also examined whether a radioreceptor assay involving the use of human placental receptors could provide a satisfactory alternative for laboratories in which antisera are not available.

A major problem in the measurement of somatomedin in human plasma samples has been the interference by somatomedin-binding proteins. Furlanetto et al. (7) attempted to overcome this by using non-equilibrium assay conditions, to permit dissociation of the somatomedin-binding-protein complex. Other approaches include acidification, lyophilization, and reconstitution (24), and gel chromatography of each sample at low pH (30). Recently a simple method has been described by Daughaday et al. (27), involving only extraction with acid–ethanol and neutralization. This method reportedly gives quantitative recovery of somatomedin, and it appears to enhance the discrimination between hypopituitary and normal samples, because the increase in immunoreactive somatomedin C on extraction was 1.6-fold for hypopituitary samples but almost threefold for normal samples (27). We find this method to be technically undemanding and of satisfactory reproducibility, and have used it on all samples described in this paper.

In its activity toward various peptides and toward extracts of normal and pathological plasma samples, antiserum Tr4 was analytically indistinguishable from the National Pituitary Agency antiserum throughout this evaluation. Because the latter antiserum is known to be specific for somatomedin C/IGF-1, we assume that antiserum Tr4 has the same specificity. Therefore the assay involving Tr4 and tracer of high somatomedin C immunoreactivity (25) may be described as a somatomedin C/IGF-1 radioimmunoassay. Final confirmation of this designation, however, will require that pure somatomedin C and IGF-1 be tested in this system.

Two differences, neither of analytical significance, were seen between the NPA RIA and the Tr4 RIA. First, the NPA RIA is approximately twice as sensitive towards both purified somatomedin and extracted normal plasma pool. Second, the Tr4 RIA showed a slight upward bias as compared with the NPA RIA in samples from normal persons and from hypopituitary and renal-failure patients, but not acromegalic patients. Despite this, there was no overlap between normal and acromegalic values in either assay. The lower values obtained for hypopituitary samples with the NPA RIA meant that five of 13 values in this study were unmeasurably low. If required, accurate values could be assigned simply by increasing the sample volume, because only 1- and 2-μL volumes were measured in the present protocol.

In contrast to the two very similar antibodies, the human placental membrane RRA was analytically worse in distinguishing growth-hormone disorders, and also showed significant biases in both the hypothryroid and the renal-failure groups. The overlap between normal and hypopituitary ranges has been observed in placental membrane assays with either IGF-1 (27) or somatomedin A (15) as tracer, and clearly indicates the value of the radioimmunoassay in this area. The twofold increase in RRA values over radioimmunoassay values that we observed in hypopituitarism is in exact agreement with the observation of Kemp et al. (31). In active acromegaly,
our lack of overlap with normal values is similar to the RRA with IGF-1 as tracer (27), but contrasts with the somatomedin A RRA of Takano et al. (15), in which some overlap was observed. However, Hall et al. (8) have described a radioimmunoassay and an RRA for somatomedin A, both of which give excellent discrimination between normals and acromegals. Although normal and acromegalic ranges did not overlap by RRA, this assay systematically gave 39% lower values for acromegals than did either radioimmunoassay. A radioimmunoassay–RRA comparison by Furlanetto et al. (32) showed a similar result.

Thyroid hormones have been implicated in the regulation of serum somatomedin in experimental animals (33, 34), so it was of interest to study a group of patients with primary hypothyroidism. Marek et al. (35) reported 20% lower somatomedin activity, as measured by a chick cartilage bioassay, in untreated hypothyroid patients than in normals, but no significant difference was seen in results by two different radioreceptor assays (15, 36). In our study, hypothyroid patients had somatomedin values 30% lower than normal by both radioimmunoassays—confirming the somatomedin C radioimmunoassay results of Furlanetto et al. (32)—and by RRA the hypothyroid values were a striking 55% lower than normal. In all assays there was overlap between hypothyroid and normal ranges, although this was least marked in the RRA. Because the RRA systematically gave values 30–40% lower than the radioimmunoassays for hypothyroid samples, it appears that peptides measured by our RRA depend more on thyroid hormone status than do those measured by radioimmunoassay. Conversely, because RRA somatomedin peptides are less dependent on somatotropin status than those measured by radioimmunoassay, the suggestion that the low somatomedin concentrations seen in hypothyroidism may be due to secondary growth-hormone deficiency (32) seems unlikely.

Chronic renal failure reportedly increases somatomedin concentrations above the normal range as measured either by RRA (15, 37) or by somatotropin A RIA (38), in contrast to the very low activity measured by bioassay (37, 39, 40). In the present study, the radioimmunoassay methods actually gave values lower than normal for renal patients before dialysis, increasing to normal on dialysis. Even the approximately 50% higher values measured by RRA did not approach the extremely high values reported for assays in which somatomedin A is used as tracer (38). A possible explanation for the discrepancy between radioimmunoassay and RRA results is that the RRA (and the radioimmunoassay with somatotropin A as tracer) might measure nondialyzable somatomedin fragments, or other unusual circulating forms, in renal failure that are not detected by the somatomedin C radioimmunoassay methods. The increase in somatomedin concentrations after dialysis, seen in all three assays in the present study, suggests that somatomedin synthesis may be inhibited by dialyzable circulating factors in renal failure—although hemococoncentration might also contribute to the increase in some patients. Whether such inhibitors of somatomedin synthesis, if they exist, are similar to the postulated inhibitors of somatomedin action (37, 40) in renal failure remains to be established.

Several conclusions may be drawn from the method comparisons presented in this paper.

First, antiseraum Tr4, like the National Pituitary Agency antisera, appears well suited for clinical use in the diagnosis and monitoring of disorders of growth hormone. With both antisera, somatomedin concentrations approaching those of hypopituitarism may be seen in patients with hypothyroidism or chronic renal failure.

Second, the placent membrane RRA seems to measure peptides that show less growth-hormone dependence, but greater dependence upon thyroid hormones, than do those measured by the radioimmunoassay methods.

Third, as evaluated by both radioimmunoassay and RRA, hemodialysis of patients with chronic renal failure causes an increase in plasma somatomedins, suggesting the removal of an inhibitor of somatomedin synthesis. Although the RRA may give results for somatomedin that are of clinical value to laboratories where an antiserum is not available, the advantages in sensitivity and specificity shown by somatomedin radioimmunoassays make them the current methods of choice for clinical use.

The work was supported by the National Health and Medical Research Council. Specific antisera to somatomedin C was provided by the National Pituitary Agency through the Pituitary Hormone Distribution Program, supported by the National Institute of Arthritis, Metabolism and Digestive Diseases and the National Institute of Child Health and Human Development, NIH. NPA also provided ovine and human somatotropin and prolactin. Rat somatotatin was generously donated by Dr. W. Daughaday. We thank Ms. M. Prancunas for expert secretarial assistance.

References


