THE EARLY ANTIBODY RESPONSE TO HUMAN GROWTH HORMONE

By

Stanley M. Warner and S. Douglas Frasier*

ABSTRACT

The development of antibodies to human growth hormone (HGH) was studied in three growth hormone deficient patients utilizing specific antisera to Ig G and Ig M immunoglobulins. At 2½ weeks after beginning growth hormone administration binding of HGH131I to Ig M (12.1–16.3%) and Ig G (8.8–11.2%) immunoglobulins was demonstrated. At 13 weeks after the onset of therapy binding to Ig M had decreased (7.2–9.4%) and the binding to Ig G had increased (11.2–15.7%). The binding of HGH131I to immunoglobulins was inhibited by unlabelled HGH. These findings, which are typical of the sequential development of antibodies of different immunoglobulin classes seen in the early antibody response, strengthen the concept that the binding of HGH131I to plasma proteins observed in response to HGH therapy represents the development of specific antibody.

The development of antibodies to human growth hormone (HGH) during treatment with HGH has been observed by several investigators (Trafford et al. 1963; Roth et. al. 1964; Prader et al. 1964; Parker et al. 1964; Frasier & Smith 1966). These reports have suggested that these antibodies are Ig G immunoglobulins (Roth et al. 1964; Prader et al. 1964; Frasier & Smith 1966).

The usual response to antigenic stimulation in both animals and man is characterized by the sequential development of antibodies of three immunoglo-
bulin classes. In general, this sequence is Ig M immunoglobulins followed by Ig G immunoglobulins (Smith & Eitzman 1964). Ig A immunoglobulins appear between these classes in some subjects. If the binding of \( \text{HGH}^{131}\text{I} \) to plasma proteins does in fact represent the development of specific antibody, investigation of the early antibody response with specific antisera to Ig M and Ig G immunoglobulins should show initial binding of \( \text{HGH}^{131}\text{I} \) to Ig M which is subsequently replaced by binding to Ig G. We have studied this aspect of the antibody response to HGH. Similar studies have not been previously reported.

**METHODS**

Plasma samples were obtained from three growth hormone deficient patients prior to HGH administration and 2\( \frac{1}{2} \), 6 and 13 weeks following the initiation of HGH therapy. Patients received 2 or 10 International Units (IU) HGH three times a week as shown in Table 1.

Plasma samples were screened, in duplicate, for the binding of \( \text{HGH}^{131}\text{I} \) to plasma proteins by the chromatoelectrophoretic method (Roth et al. 1964) as previously described (Frasier & Smith 1966). Incubation mixtures consisted of 100 \( \mu \)l plasma, 100 \( \mu \)l \( \text{HGH}^{131}\text{I} \) and 800 \( \mu \)l 0.05 M barbital buffer (pH 8.6) containing human serum albumin 2.5 mg/ml. Final plasma dilution was 1:10. All samples were screened simultaneously utilizing a single preparation of \( \text{HGH}^{131}\text{I} \). The percentage of \( \text{HGH}^{131}\text{I} \) bound to plasma proteins in pretreatment plasma samples was used to correct for non-specific binding (Yalow & Berson 1960).

**Table 1.** Study Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Years-Months</th>
<th>Diagnosis</th>
<th>HGH*</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td>10-1</td>
<td>Craniopharyngioma Panhypopituitarism</td>
<td>E-671**</td>
<td>2 IU ( 5.6 mg) t. i. w.</td>
</tr>
<tr>
<td>MP</td>
<td>14-6</td>
<td>Isolated GH Deficiency</td>
<td>R-20***</td>
<td>2 IU ( 2.5 mg) t. i. w.</td>
</tr>
<tr>
<td>TK</td>
<td>8-6</td>
<td>Isolated GH Deficiency</td>
<td>R-20</td>
<td>10 IU (12.5 mg) t. i. w.</td>
</tr>
</tbody>
</table>

* HGH provided by the National Pituitary Agency, National Institute of Arthritis and Metabolic Diseases through the Collaborative Growth Hormone Treatment Project.

** HGH # E-671, extracted from embalmed pituitary glands, has a biologic potency of 0.36 IU/mg.

*** HGH # R-20, extracted from unembalmed pituitary glands, has a biologic potency of 0.8 IU/mg.
Studies of the binding of HGH\textsuperscript{131}I to Ig M and Ig G immunoglobulins were performed utilizing immunoprecipitation (Feinberg 1954). The precipitating antibody was either rabbit anti-human Ig M or rabbit anti-human Ig G serum (Hyland Laboratories). A single lot of each antiserum was used in all studies. Antisera were tested for monospecificity by microimmunoelectrophoresis (Scheidegger 1955) against pooled normal human serum. A single precipitin line was observed with each antiserum.

Preliminary antigen-antibody titrations were performed to determine the conditions for maximum precipitation after addition of anti-Ig M or anti-Ig G serum. Each plasma sample was titrated separately. Titration mixtures contained 25 µl test plasma, varying amounts of either anti-Ig M or anti-Ig G serum and 0.05 m barbital buffer to bring the final volume to 1 ml. Replicate mixtures were incubated 24, 48 and 72 hours at 4°C. Precipitates were separated by centrifugation, washed twice with 10 ml 0.05 m barbital buffer and dissolved in 4 ml 0.25 m acetic acid. The protein concentration of acetic acid solutions was determined by measuring absorption at 278 nm (Gitlin 1949). The optimal amounts of added antiserum varied from 160–200 µl of anti-Ig M and from 160–200 µl of anti-Ig G for different plasma samples. The volume of antiserum shown to give maximum precipitation with a given plasma sample was used in subsequent studies of that plasma. Maximum precipitation was observed after 48 hours of incubation.

Plasmas were tested in duplicate for determination of the binding of HGH\textsuperscript{131}I to specific immunoglobulins. All samples were tested simultaneously with a single HGH\textsuperscript{131}I preparation. Initial incubation mixtures contained 25 µl plasma, 100 µl HGH\textsuperscript{131}I and either 375 µl 0.05 m barbital buffer or 100 µl unlabelled HGH (5 µg/ml) and 275 µl 0.05 m barbital buffer. Final plasma dilution was 1:20. After incubation at 4°C for five days, the appropriate volume of antiserum and sufficient 0.05 m barbital buffer to give a final volume of 1 ml were added. After an additional 48 hours at 4°C, precipitates were separated by centrifugation. Precipitate (bound) and supernatant (free) radioactivity were determined separately in a well-scintillation counter.

**RESULTS**

When tested by chromatoelectrophoretic method, the binding of HGH\textsuperscript{131}I to plasma proteins varied from 3.8 – 7.8 % at 2½ weeks and from 11.7 – 16.7 % at 13 weeks.

The percentage HGH\textsuperscript{131}I bound to Ig M and Ig G immunoglobulins is shown in Table 2 and Table 3, respectively. Binding at each time of sampling was compared to pretreatment binding by t test for paired observations. There was significant binding of HGH\textsuperscript{131}I to Ig M immunoglobulins 2½ weeks after beginning HGH administration. Binding to Ig M disappeared over the period of observation. Significant binding of HGH\textsuperscript{131}I to Ig G immunoglobulin was observed at each time of sampling and increased slightly over the period of observation. The addition of excess unlabeled HGH completely inhibited the binding of HGH\textsuperscript{131}I to both Ig M and Ig G immunoglobulins in plasma samples obtained after initiation of treatment. The binding observed in control plasma samples was not inhibited by the addition of unlabeled HGH.
Table 2.
Per cent HGH\textsuperscript{131}I Bound to Ig M Immunoglobulin.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time (Weeks)</th>
<th>0</th>
<th>2(\frac{1}{2})</th>
<th>6</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td></td>
<td>7.7</td>
<td>16.3</td>
<td>10.5</td>
<td>7.2</td>
</tr>
<tr>
<td>MP</td>
<td></td>
<td>7.2</td>
<td>12.1</td>
<td>11.4</td>
<td>7.8</td>
</tr>
<tr>
<td>TK</td>
<td></td>
<td>6.8</td>
<td>12.9</td>
<td>7.3</td>
<td>9.4</td>
</tr>
<tr>
<td>Mean ± sd</td>
<td></td>
<td>7.2 ± 0.5</td>
<td>13.8 ± 2.2</td>
<td>9.7 ± 2.2</td>
<td>8.1 ± 1.1</td>
</tr>
</tbody>
</table>

- \(t (2\frac{1}{2} \text{ weeks vs control}) < 0.05\)
- \(t (6 \text{ weeks vs control}) \text{ N.S.}\)
- \(t (13 \text{ weeks vs control}) \text{ N.S.}\)

Table 3.
Per cent HGH\textsuperscript{131}I Bound to Ig G Immunoglobulin.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time (Weeks)</th>
<th>0</th>
<th>2(\frac{1}{2})</th>
<th>6</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS</td>
<td></td>
<td>6.0</td>
<td>8.8</td>
<td>12.9</td>
<td>15.7</td>
</tr>
<tr>
<td>MP</td>
<td></td>
<td>6.1</td>
<td>11.0</td>
<td>13.6</td>
<td>11.2</td>
</tr>
<tr>
<td>TK</td>
<td></td>
<td>5.9</td>
<td>11.2</td>
<td>11.0</td>
<td>14.8</td>
</tr>
<tr>
<td>Mean ± sd</td>
<td></td>
<td>6.0 ± 0.1</td>
<td>10.3 ± 1.3</td>
<td>11.7 ± 1.0</td>
<td>13.9 ± 2.4</td>
</tr>
</tbody>
</table>

- \(t (2\frac{1}{2} \text{ weeks vs control}) < 0.05\)
- \(t (6 \text{ weeks vs control}) < 0.02\)
- \(t (13 \text{ weeks vs control}) < 0.05\)

COMMENTS

Several lines of evidence indicate that the binding of radioiodinated human growth hormone (HGH\textsuperscript{131}I) to plasma proteins which has been observed in patients receiving human growth hormone (HGH) therapy represents the development of specific antibodies to HGH. Specific binding is uniformly absent in pretreatment samples and decreases after cessation of therapy (Traf-ford et al. 1963; Roth et al. 1964; Prader et al. 1964; Parker et al. 1964; Frasier & Smith 1966). An anamnestic response has been observed in subjects receiving
a subsequent course of HGH (Roth et al. 1964; Prader et al. 1964; Parker et al. 1964). Binding of HGH$^{131}$I is inhibited by unlabelled human growth hormone (Roth et al. 1964; Prader et al. 1964; Parker et al. 1964; Frasier & Smith 1966), simian growth hormone (Parker et al. 1964; Frasier & Smith 1966; Tashjian et al. 1966) and human placental lactogen (Parker et al. 1964), but not by bovine growth hormone (Prader et al. 1964; Frasier & Smith 1966) or ovine growth hormone (Frasier & Smith 1966). Neutralization of the clinical response to HGH has been reported in five patients whose plasma or serum proteins bind HGH$^{131}$I (Prader et al. 1964; Parker et al. 1964; Frasier & Smith 1966). Neutralization of tibial cartilage response to HGH in hypophysectomized rats by plasma which was shown to bind HGH$^{131}$I has also been observed (Frasier & Smith 1966).

The findings reported in this study strengthen the concept that binding of HGH$^{131}$I to plasma proteins observed in association with HGH administration represents the formation of specific antibody. Through the utilization of specific antisera to human Ig M and Ig G immunoglobulins these studies have shown binding of HGH$^{131}$I to both Ig M and Ig G immunoglobulin 2½ weeks after initiating HGH therapy. At 13 weeks, binding of Ig M had disappeared while significant binding to Ig G persisted. This sequence of antibody formation involving different classes of immunoglobulins is characteristic of the early response to antigenic stimulation (Smith & Eitzman 1964).

The development of antibodies in man to a protein derived from human sources remains to be explained. The most likely explanation for the antigenicity of HGH in man is that the HGH molecule is altered during preparation and that certain individuals are immunologically intolerant to the altered protein. An alternative explanation is an alteration of the HGH molecule at the site of injection. The observation that an antibody response is shown by only certain individuals raises the possibility of antigenic differences between the endogenous growth hormone of different individuals. A particular growth hormone preparation which is derived from a pool of pituitaries may contain growth hormones which are antigenic for certain individuals but not for others receiving the same preparation.

**SUMMARY**

The early antibody response to the administration of HGH has been studied in three growth hormone deficient patients. Employing immunoprecipitation with specific anti-human Ig M and Ig G sera, early binding of HGH$^{131}$I to Ig M immunoglobulin and subsequent binding to Ig G immunoglobulin has been demonstrated. These findings strengthen the concept that binding of HGH$^{131}$I to plasma proteins in patients treated with HGH represents specific antibody.
REFERENCES


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