Quantitative Differences between Immunological and Receptor Binding Activities of hGH in Pituitary Adenomas from Patients with Acromegaly

NAOMI HIZUKA, KAZUE TAKANO, KAZUO SHIZUME, KUMIKO ASAKAWA AND MEGUMI MIYAKAWA

Department of Medicine, Institute of Clinical Endocrinology, Tokyo Women's Medical College, Tokyo 162

Abstract

Human growth hormone (hGH) in the pituitary extracts from patients with acromegaly and normal subjects was measured by radioimmunoassay (RIA) and radioreceptor assay (RRA) using pregnant rabbit liver membrane, and the RRA to RIA ratios of hGH were calculated. The RRA to RIA ratio of hGH for acromegaly was higher than that for normal (0.90±0.03 vs 0.75±0.03, p<0.01). When the pituitary extracts were gel filtered on Sephadex G-100 column there were three immunoreactive hGH peaks ("pre-big", "big", and "little" hGH); there were no significant differences in gel filtration patterns of hGH between acromegaly and normal. The "little" hGH component (major component) had a higher RRA to RIA ratio than "pre-big" hGH and "big" hGH in both acromegaly and normal. "Little" hGH extracted from acromegaly had a higher RRA to RIA ratio than that from normal (1.14±0.09 vs 0.93±0.04, p<0.05).

These data support the hypothesis that hGH synthesized in acromegaly may be different from normal.

Human growth hormone (hGH) has been shown to be heterogenous in plasma with respect to molecular size (Goodman et al., 1972; Gorden et al., 1973; Lewis et al., 1980). Further the major component, "little" or 22K hGH, has been shown to have a high receptor-reactive to immunoreactive potency in plasma from acromegalic subjects than normal subjects (Gorden et al., 1976).

Until now these differences in hGH between normal and acromegalic subjects have been studied only in plasma and it has not been known whether these differences are native to the pituitary gland or produced following secretion.

In the present study we have taken advantage of fresh pituitary extracts to show that the qualitative difference in normal and acromegalic hGH has its origin in the pituitary gland.
Materials and Methods

Hormone

Immunological grade hGH preparation (AFP-4793B) was a gift from National Hormone and Pituitary Program, NIADDK, NIH. The hGH was used as standard in both radioimmunoassay (RIA) and radioreceptor assay (RRA), and also used for iodination. hGH was iodinated to a specific activity of 30–40 μCi/μg by a modification of chloramine-T method (Lesniak and Roth 1976).

Pituitary tissues and extraction of hGH

Pituitary tissues were obtained from adenomas of seven patients with acromegaly by surgery. Normal pituitary tissues were obtained from ten patients without endocrine disease at autopsy between three and five hours post mortem. The pituitary tissues were frozen on dry ice immediately after removal, and kept at −70°C until extraction.

The tissues were homogenized in normal saline and the homogenate was centrifuged 20,000×g for 45 min at 4°C as described by Singh et al. (Singh et al., 1974). The supernatant, designated as pituitary extract, was used for measurement of hGH.

The pituitary extracts were filtered on a Sephadex G-100 column (1.5×80 cm) equilibrated in and eluted with 0.05M ammonium bicarbonate at 4°C. Two milliliter fractions were collected, and each fraction was assayed directly by RIA and RRA.

RIA and RRA of hGH

RIA using double antibody technique was carried out. Rabbit anti-hGH serum (AFP-97720133) was kindly provided by the National Hormone and Pituitary Program, NIADDK, NIH.

RRA using pregnant rabbit liver membrane was performed as described previously (Pavlakis et al., 1981; Hizuka et al., 1982). Briefly, the membrane preparation (200 μg protein) was incubated with 125I-hGH (0.25 ng/ml) and unlabeled hGH (0–1,000 ng/ml) or a sample in a total volume of 0.5 ml in polyethylene microfuge tubes at 4°C for 16 hours. At the end of the incubation, the tubes were centrifuged for 4 min in a Beckman microfuge. The supernatant was aspirated, the membrane pellet at the tip of the tube was excised, and the radioactivity in the pellet was counted.

The ratio of receptor-reactive hGH to immunoreactive hGH (RRA to RIA ratio) was calculated.

Statistics

Student’s t-test was used for statistical analysis.

Results

RRA to RIA ratio of hGH in pituitary extracts

The hGH in pituitary extracts from seven patients with acromegaly and ten normal subjects were measured by RRA and RIA for hGH, and RRA/RIA was calculated. The horizontal lines represent the mean values.

![Fig. 1. RRA to RIA ratio (RRA/RIA) of hGH in pituitary extracts. The pituitary extracts from seven acromegalic and ten normal subjects were measured by RRA and RIA for hGH, and RRA/RIA was calculated. The horizontal lines represent the mean values.](image-url)
normal subjects was measured by RRA and RIA, and the RRA to RIA ratios were calculated (Fig. 1). The mean RRA to RIA ratio for acromegaly was 0.90±0.03 and that for normal was 0.75±0.03. The ratio for acromegaly was significantly higher than that for normal (p<0.01).

**Gel filtration pattern of hGH**

When the pituitary extract was gel filtered on a Sephadex G-100 column, there were three immunoreactive hGH peaks (Fig. 2). The major component, “little” hGH, eluted as an approximately 22K protein.

![Gel filtration pattern of hGH](image)

**Table 1.** Proportion of “pre-big”, “big”, and “little” hGH components in pituitary extracts from acromegaly and normal.

<table>
<thead>
<tr>
<th></th>
<th>“pre-big”</th>
<th>“big”</th>
<th>“little”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acromegaly</td>
<td>4.3±0.7%</td>
<td>11.2±0.8%</td>
<td>84.5±1.1%</td>
</tr>
<tr>
<td>Normal</td>
<td>7.4±2.4%</td>
<td>15.7±1.9%</td>
<td>76.9±4.6%</td>
</tr>
</tbody>
</table>

![RRA to RIA ratio of “little” hGH](image)
In addition, higher molecular weight components were found consisting of an approximately 45K protein, “big” hGH, and higher molecular component, “pre-big” hGH. The gel filtration pattern of immunoreactive hGH in pituitary extracts from seven patients with acromegaly and nine normal subjects were investigated (Table 1). The percentage of “little” hGH in total immunoreactive hGH for acromegaly was slightly higher but not significantly than that for normal. There were no significant differences in the proportions of both “big” and “pre-big” hGH between acromegaly and normal.

RRA to RIA ratios of hGH in “little”, “big”, and “pre-big” hGH

We next studied the RRA to RIA ratios of hGH in “little”, “big”, and “pre-big” hGH components in the pituitary extracts. The fractions of each component were pooled and were measured by RRA and RIA. The mean of RRA to RIA ratio for “little” hGH in pituitary extracts from seven patients with acromegaly was 1.14 ± 0.09 and that for eight normal subjects was 0.93 ± 0.04 (p < 0.05) (Fig. 3). The RRA to RIA ratios for “big” and “pre-big” hGH are shown in Figure 4. Compared to “little” hGH, the ratios in “big” and “pre-big” hGH for both acromegaly and normal were quite low. There were no significant differences between the ratios in “big” and “pre-big” hGH for acromegaly and those for normal.

It is interesting to note that one patient with active acromegaly, whose plasma hGH found by RIA was 5 ng/ml, had the highest RRA to RIA ratios in whole pituitary extract (1.03), and “little”, “big”, and “pre-big” hGH (1.63, 0.95, 2.15), respectively. In addition her plasma somatomedin-C value was high (3.13 U/ml).

Discussion

In the present study we found that the RRA to RIA ratios of hGH in whole pituitary extract and the “little” hGH component

![Fig. 4. RRA to RIA ratio of “pre-big” and “big” hGH in pituitary extracts from patients with acromegaly and normal subjects. The horizontal lines represent the mean values.](image-url)
from patients with acromegaly were higher than those from normal, and that the RRA to RIA ratios of hGH for the higher molecular weight components of hGH ("pre-big" and "big" hGH) in pituitary extracts from both acromegaly and normal were lower than those for "little" hGH.

The finding that the hGH produced in acromegaly had higher receptor binding activity than that for normal is similar to that observed in plasma hGH by Gorden et al. (1976). By contrast, Rosenfeld and Hintz (1980) and Gavin et al. (1982) did not find any differences in receptor binding activity of plasma hGH between acromegaly and normal. These different results might be explained in part by the different anti-hGH antibody and hGH standard used in the assays. In our study the mean RRA to RIA ratios of hGH in acromegaly were statistically higher than those of normals, but in some acromegalic patients these ratios were not different from normal. It is to be noted, however, that one patient with active acromegaly whose plasma hGH found by RIA was only 5 ng/ml, had the highest RRA to RIA ratio of hGH in the pituitary extract. In this case it is reasonable to suppose that the higher receptor-reactive hGH is responsible for these clinical manifestations.

Recent recombinant DNA technology has shown that the human genome contains five hGH related genes (Barsh et al., 1983). One of these, designated hGH 2 gene or hGH-V gene, encodes a variant hGH peptide which differs by 13 amino acids from authentic hGH (hGH 1 or hGH-N) (Seeburg 1982). When the hGH 2 gene is expressed in monkey kidney cells, this peptide has a higher RRA to RIA ratio of hGH than hGH 1 (Pavlakis et al., 1981; Hizuka et al., 1982). Whether this variant hGH is produced in the pituitary is not known, but mixtures of variant hGH and authentic hGH could explain our findings in this study.

Briefly, this study provides a direct demonstration that pituitary hGH from some acromegalic patients has greater receptor-reactivity than immunoreactivity in comparison with hGH from normal pituitary.

Acknowledgements

The authors thank Dr. Phillip Gorden, NIH, Bethesda, for his kind suggestion and revision of the manuscript, Dr. Kageyama and Dr. Kuwayama, Nagoya University, for providing the tissues of pituitary tumors, and Dr. Imai, Tokyo Women's Medical College, for providing the normal pituitary tissues. The authors also thank Miss S. Hasegawa and Miss S. Saito for their excellent technical assistance.

This work was partly supported by Grants in Aid for Scientific Research, Ministry of Education Science and Culture, Japan, (Nos. 58771756, 58571012 and 58440084), a research grant from the Intractable Diseases Division, Public Health Bureau, Ministry of Health and Welfare and a research grant from the Foundation for Growth Science in Japan.

References


