Studies on the Heterogeneity of Growth Hormone in Human Pituitary

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Synopsis

The presence of three different growth hormones (GH) in molecular weight, “big big GH”, “big GH” and “little GH”, is reported here. Human pituitary extracts from an acromegalic patient and from a postmortem patient were fractionated into three fractions using gel filtration of Sephadex G-200. The major component of the pituitary GH was observed at the most retarded fraction (“little GH”), which was identical with the highly purified HGH in the elution pattern. The “big GH” was less retarded in the gel filtration and was eluted between the “little GH” and 4S fraction. The small peak of GH with the largest molecular weight (“big big GH”) was eluted between 19S and 7S fractions. The activity of these three GHs in different molecular weights was observed using GH radioimmunoassay. In refiltration of each fraction of normal human pituitary extract, these three components were isolated independently at each filtration. The conversion from “big big GH” to “big GH” or to “little GH” was observed during storage at 4°C for 7 days and the similar conversion, from “big GH” to “little GH” was also observed in refiltration of “big GH”, however the reverse reaction from “little GH” to “big GH” was not observed. The physiological significance of these hormones of different molecular weights is not clear, but it is suggested that the immunoreactive GH with large molecular weight may be a form of the precursor or polymer.

Materials and Methods

Preparation of pituitaries

A human pituitary, which was obtained from a patient with normal GH response (within 6 hrs after
death), was kept frozen before use. The pituitary was homogenized in 0.01 M phosphate buffer, pH 7.8 at 4°C and centrifuged at 100,000 x g for 60 min. The supernatant of the homogenate was applied to gel filtration immediately in parts, and the remainder was kept frozen for subsequent use. A part of the pituitary tissue (acidophil adenoma) from an acromegalic patient was obtained during surgical resection and was treated in the same way.

**Sephadex gel filtration**

Nonacromegalic pituitary extracts or acromegalic pituitary extracts were subjected to gel filtration on Sephadex G-200 (Pharmacia) on column 1.3 x 170 cm at 4°C using 0.01M phosphate buffer, pH7.8. Effluents were collected in fractions of 5 ml volume at a flow rate of 10-20 ml per hr. On refiltration of each subfraction, freshly isolated, stored or freeze-dried subfraction was applied individually to the same column with pooled human serum (NHS) of 0.5 ml. Protein concentration was measured with an optical density at 280 nm of Hitachi Perkin-Elmer 139 UV-Vis spectrophotometer.

**GH radioimmunoassay**

Following gel filtration, the effluents were kept at 4°C until assayed. GH activity was determined by a double antibody radioimmunoassay (RIA) using HGH kits of Dainabot laboratory. The diluted or undiluted effluent (in amounts of 0.1, 0.5 or 1.0 ml) was used for RIA. The final volume in the assay system was the same amount as in the standard assay system.

**Results**

When the supernatant of the pituitary extract from an acromegalic patient was subjected to gel filtration on Sephadex G-200, three peaks of GH immunoreactivity were detected (Fig. 1). The major peak of immunoreactive GH (IRGH) coincided with the position of 125I-HGH which was used to calibrate the elution. Close to the void volume, two small peaks of IRGH were observed. For the purpose of identification of these peaks of IRGH, the supernatant of the pituitary extract from a nonacromegalic patient was applied to gel filtration on Sephadex G-200 as shown in Figure 2 (upper left). The major peak of IRGH was seen in the retarded fraction after 4S. Small and broad peaks were seen between the void volume and the major IRGH peak. Three tubes of the original effluents, called A, B and C fraction in Figure 2 (upper left) were refiltrated. Each original effluent was refiltrated freshly with NHS twice without freezing, and three isolated peaks of each fraction were noticed in the same regions as illustrated in Figure 2. Following the results of the fractional analysis of NHS proteins, three major fractions were separated by gel filtration and identified as 4S fraction (mainly albumin), 7S fraction (mainly r-globulin) and 19S fraction (mainly macroglobulin) (Fahey and Terry, 1968). In the present studies, these three protein fractions were employed for orientation of the elution pattern. The major peak of IRGH refiltrated from the A fraction of original effluent was noticed between 19S fraction and 7S fraction of NHS and this component is designated as "big big GH". The major peak of IRGH refiltrated from the B fraction of the original effluent was located in the area just after 4S fraction and in the area between 4S fraction of NHS and the peak of purified HGH. The IRGH in this fraction was proposed as "big GH" by Goodman et al. (1972) and Gorden et al. (1972). The most retarded peak with small molecular weight which was refiltrated from the C fraction of originated effluent, was proposed as "little GH" by previous investigators (Frohman et al., 1972; Goodman et al., 1972; Gorden et al., 1972). The "little GH" coincided with the peak of 125I-HGH of purified GH and was retarded from "big GH" in filtration. In these patterns of refiltrations it is evident that three GHs with different molecular weight were detectable in the human pituitary and were immunologically active.

In refiltration of the isolated "big big GH", "big GH" and "little GH" stored at 4°C for 7 days, smaller IRGHs in molecular weight appeared in effluents. Figure 3 indicates the amounts of IRGH in refiltration of the isolated "big big GH" after storage at 4°C for 7 days. A decreased amount of "big big GH" was seen, and smaller IRGHs in molecular
Fig. 1. Sephadex G-200 gel filtration pattern of pituitary extract from an acromegalic patient (acidophil adenoma). Two ml of the supernatant of pituitary extract was filtered. IRGH measured by HGH RIA is expressed by the shaded area. Protein concentration is described by the solid line (hemolized extract shows its peak at Hb level).

Fig. 2. Isolation of “big big GH”, “big GH”, and “little GH”. Upper left: Gel filtration pattern of pituitary extract (2 ml) from a post-mortem patient. IRGH is expressed by the shaded area. Protein concentration is shown by a solid line. Refiltration of tube A of “big big GH” peak (bottom left), tube B of “big GH” peak (upper right) and tube C of “little GH” peak (bottom right) are shown in one peak at each re-filtration. In refiltration 1.5 ml of each eluate (tube A, B or C) and 0.5 ml of NHS were applied on the fraction column. The eluted pattern of NHS showed three major peaks indicated by a solid line. The first peak from the left was noticed as 19S fraction, the second peak as 7S and the third peak as 4S. The last fraction might be polypeptide or free amino acids.
weight, such as “big GH” and “little GH”, were detected in effluents. In refiltration of the isolated “big GH” stored at 4°C for 7 days, decreased activity of “big GH” and the appearance of “little GH” were seen in gel filtration (Fig. 4). On the contrary, the refiltration of the isolated “little GH” stored at 4°C for 7 days, exhibited only ‘little GH’ in less activity and did not contain the bigger GH in molecular weight.

The characteristics of the immunoreactivity of these GHs at different molecular weights were studied using the binding activity of GH with an identical anti-GH antibody in different dilutions of each GH, as shown in Figure 5. The slope patterns of “little GH” and “big GH” were almost identical with the purified GH; however, the slope of “big big GH” was slightly different with a lower binding percent at one eighth dilution as compared with the binding percent of the other GHs at one eighth dilution.

Discussion

Goodman et al. (1972) and Gorden et al. (1972) found the presence of “big GH” with a molecular size twice bigger than that of “little GH” by gel filtration of plasma obtained from patients with acromegaly and normal subjects. Similar observations of “big GH” were reported in fractionation of pituitary extracts (Frohman et al., 1972; Gorden et al., 1972). However the large molecular sized GH observed by Frohman et al. (1972) was eluted just after the void volume, which might be almost compatible with our “big big GH” in the elution pattern. The “big GH” by Goodman et al. (1972) and Gorden et al. (1972) was almost identical with our “big GH” in filtration pattern.

The existence of three different forms of insulin and gastrin stimulated us to investigate the new form of HGH in the plasma and pituitary. The “big big GH” is located in size between 19S fraction and 7S fraction, and
Fig. 5. Dilution patterns (slopes) of purified GH, "little GH", "big GH" and "big big GH" by RIA.

has an immunoreactivity against anti-"little GH" antibody. However there exists only a little activity of IRGH between the fraction of "big big GH" and "big GH" in Figure 2. These IRGH of high molecular weights might be the intermediate sized forms between "big big GH" and "big GH". Recently Stachura and Frohman (1974) reported the existance of intermediate sized GH forms using rat pituitary. By twice repeated refractionation, "big big GH" was enriched to about 65% of total filtered IRGH. In the process of storage, the "big big GH" showed progressive loss in immunoreactivity similar to "big big insulin" (Yalow and Berson 1972). Therefore, the refiltration should be run immediately after previous elution.

Freezing and thawing of solution of $^{125}$I-HGH in phosphate buffer promoted the conversion of monomeric GH to high molecular weight material (Schwartz and Batt 1973). Actually, in the present study the pituitary gland was kept frozen at $-20^\circ$C for a few months before use. Therefore, it may not be denied that the materials with high molecular weights in effluents, converted from monomeric GH by freezing, can exist and that the peptide hormones denature and show some aggregation during the iodination process. On the contrary, the present study indicated the conversion from "big big GH" to "big GH" or "little GH" during storage at 4°C for 7 days. The conversion from "big GH" to "little GH" was also reported by others in 8–33% of "big GH" with prolonged storage (Gorden et al. 1972), in more than half of "big GH" during storage at $-20^\circ$C(Goodman et al. 1972) and also by lyophilization (Goodman et al., 1972; Gorden et al., 1972). Of course, the immunoreactivity of "big big GH" decrease following the storage, freezing and lyophilization in the present study. These conversion from "big big GH" to "big GH" or "little GH" may suggest that the "big big GH" is a polymerized form or a physiological precursor of the smaller form of GH. However, the possibility that "big big GH" and "big GH" are a polymer or a precursor of "little GH" can not be confirmed nor excluded definitely at present. Another suggestion that "big big GH" and "big GH" are bound to some dissociable moiety, might be considered as suggested by Goodman et al. (1972). Stachura and Frohman (Stachura and Frohman, 1974) also suggested the possibility that "large GH", described as "big big GH" in this paper, might represent the GH polysome.

The "big GH" and "little GH" showed an immunologic similarity in RIA. However
the findings of different gradients of bound % at each dilution between “big big GH” and “big GH” or “little GH” suggest some dissimilarity of immunological activity of “big big GH” against that of “big GH” or “little GH”. Another suggestion is that the increase of total IRGH activity may occur during the incubation period of “big big GH” RIA due to conversion from “big big GH” to “little GH”. In this assumption, the immunological activity of “big big GH”, “big GH” and “little GH” may not differ from each other and a different gradient in Figure 5 may result from the different rate of conversion and also the amount of IRGH activity. In Frohman’s report (1972), no immunologic dissimilarity was observed between his “large GH” and “small GH”.

It is important to analyse the nature and the physiological role of GH of different molecular weight. In the present study, the presence of three different molecular substances of HGH in normal and acromegalic pituitary glands was demonstrated. The physiological significance and the chemical nature of these three different molecular sized GHs are unclear at present. The correlation between the immunoreactivity of these three GHs and the biological activity and the physiological requirement of these “big big GH” and “big GH” should be analysed in the future.

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References