Biological Activities of Synthesized 20K and 22K hGH in Nb2 Bioassay and IM-9 Radioreceptor Assay

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Abstract

We investigated the bioactivities of the recombinant DNA-derived methionyl 20K hGH (20K-Met-hGH) and methionyl hGH (22K-Met-hGH). The growth-promoting activities in Nb2 cells of 20K-Met-hGH and 22K-Met-hGH were 10.7% and 93.5% of pituitary hGH (P-hGH), respectively. In the IM-9 lymphocyte assay, the binding activities of 20K-Met-hGH and 22K-Met-hGH to hGH receptor were 29.0% and 87.1% of P-hGH, respectively. Our data demonstrate that 20K-Met-hGH may have weaker biological potency than P-hGH.

A variant form of human GH (hGH), termed 20,000-dalton hGH (20K hGH) is characterized by deletion of amino acid sequence 32-46 of native hGH (22 K hGH) (Lewis et al., 1978; Lewis et al., 1980). The biological activity of naturally occurring 20K hGH has been tested in various bioassays (Lewis et al., 1978; Spencer et al., 1981; Closset et al., 1983).

Nb2 cells, a rat lymphoma cell line, provide a specific and sensitive bioassay for lactogenic hormones such as prolactin and hGH. The activity of lactogenic hormones is linearly associated with cell proliferation in the Nb2 cell line (Tanaka et al., 1980). Human lymphocyte IM-9 cells have highly specific hGH receptors (Smal et al., 1985). Both cell lines have been used in bioassays and receptor assay for hGH.

In the present study, we attempted to determine the bioactivities of biotechnologically synthesized methionyl 20K hGH and methionyl 22K hGH and compare them with that of pituitary hGH, using these cell lines.

Materials

The recombinant DNA-derived 20,000-dalton variant of N-terminal methionyl hGH (20K-Met-hGH) and recombinant DNA-derived 22,000-dalton N-terminal methionyl hGH (22K-Met-hGH) were synthesized by the methods described by Ikehara et al. (1984) and Miyamoto. (1987). The pituitary hGH (P-hGH) was provided by NIH (iodination grade AFP 4793-B) and used...
for radioiodination and the standards. The antibody for RIA was provided by Prof. Friesen, Department of Physiology, University of Manitoba.

**Methods**

* Nb2 bioassay (Tanaka et al., 1980): Passaged Nb2 cells were planted at a density of 1 x 10^5 cells/well (24-well NUNC dish) using Fischer’s medium supplemented with 10% horse serum, penicillin (50 U/ml), and streptomycin (50 µg/ml). The test materials were diluted in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA), and added to each well. After 96-hour incubation, the cells were counted with a Coulter Counter.

* IM-9 radioreceptor assay: The IM-9 RRA procedure described by Rosenfeld et al. (1980) was used with minor modification. The IM-9 cells which were serially passaged in RPMI medium with 10% fetal calf serum, penicillin (50 U/ml), and streptomycin (50 µg/ml) were sedimented and resuspended in 100 mM Hepes buffer (pH 7.0) containing 1% bovine serum albumin, 120 mM NaCl, 1.2 mM MgSO4, 2.5 mM KCl, 15 mM Na acetate, 10 mM dextrose and 1 mM EDTA at a concentration of 40-50 x 10^6 cells/ml. Aliquots containing 450 µl of this cell suspension were placed in 12 x 75 mm glass tubes in duplicate, to which was added 50 µl of unlabelled 20K-Met-hGH, 22K-Met-hGH or P-hGH. 200,000 cpm of 125I-P-hGH was added and the cells were incubated for 100 minutes at 37°C. After incubation, the cells were washed with 3 ml of Hepes buffer and resuspended in 500 µl of the same buffer. Aliquots containing 450 µl were layered over 2.5 ml of iced PBS and centrifuged. Supernatants were aspirated and pellets were counted.

* Radioimmunoassay: The standard double antibody RIA method was used to determine the immunoactivity of hGH (Friesen and Carr, 1976). The same tracers and the standards as for IM-9 RRA were used.

**Results**

The growth of Nb2 cells is stimulated by supplemented hGH dose dependently (Fig. 1). 22K-Met-hGH was as potent as P-hGH, while 20K-Met-hGH weakly promoted cell proliferation. In the present study, the growth-promoting activities of 20K-Met-hGH and 22K-Met-hGH were 10.7% and 93.5% of P-hGH, respectively.

The results for IM-9 RRA are shown in Fig. 2. 20K-Met-hGH and 22K-Met-hGH inhibited the binding of 125I-P-hGH by 20K-Met-hGH poorly promotes cell proliferation at any density.
to receptors in IM-9 cells 29.0% and 87.1% of P-hGH, respectively. Immunoactivities of 20K-Met-hGH and 22K-Met-hGH were 44.6% and 96.7% of standard P-hGH, respectively, by RIA (Fig. 3).

**Discussion**

The IM-9 cell line is known to have hGH receptors on the plasma membrane. It has been shown that the reduction in the binding of \(^{125}\text{I}-\text{hGH}\) is dependent on the concentration of growth hormone present as well as the duration and the exposure (Lesniak et al., 1976). Therefore, the IM-9 cell line provides a specific measure of biologically active hGH in terms of receptor activity.

The Nb\(_2\) bioassay is based on the stimulation of the growth of Nb\(_2\) rat lymphoma cell cultures by the lactogenic hormones. It has been shown that the Nb\(_2\) cell line has prolactin receptors (Shiu et al., 1983). Though the Nb\(_2\) bioassay has been primarily used for the measurement of the bioactivity of prolactin and chorionic somatomammotropin (Tanaka et al., 1980; Tanaka et al., 1982), it could also be used for the bioassay of hGH, since hGH is known to have lactogenic activity. The Nb\(_2\) bioassay however, does not reflect the somatotropic, but only the lactogenic activity of hGH.

The differences between the biological activities of natural 20K hGH and 22K hGH in various bioassays [weight gain assay (Lewis et al., 1978), pigeon crop assay (Lewis et al., 1978), tibia width assay (Spencer et al., 1981; Closset et al., 1983) and somatomedin production test (Spencer et al., 1981)] have been reported to lack statistical significance. But the insulin-like activity of 20K hGH has been reported to be absent or weak (Frigeri et al., 1979; Kostyo et al., 1985). The receptor reactivity of natural 20K hGH was reported to be about 30% of the standard hGH in IM-9 RRA (Hizuka et al., 1982). And natural 20K hGH has a diminished activity
for suppression of free fatty acids in GH deficient children as compared to natural 22K hGH (Culler et al., 1986).

In the present study, the lactogenic activity was much less than the receptor activity of 20K-Met-hGH, compared with P-hGH as a standard. Natural 20K variant, however, has been reported to have only about 30% of the lactogenic activity of P-hGH in stimulating DNA synthesis of Nb₂ cells (Emoto et al., 1987). The difference between this report and ours could be due to the biochemical differences between the pure 20K hGH variant and its methionane derivative.

Our results by IM-9 RRA were compatible with the report by Hizuka et al. (1982). Therefore, both natural 20K hGH and 20K-Met-hGH may have diminished receptor potency.

Immunological potency was also diminished in both 20K-Met-hGH (44% of natural hGH) and natural 20K variant (30% of natural hGH) (Hizuka et al., 1982). The slight difference might be caused by the use of different antisera.

In contrast to this, 22K-Met-hGH was equipotent to P-hGH in both Nb₂ bioassay and IM-9 RRA. Therefore, in 22K hGH, added methionine might play little part in lactogenic activity, receptor binding activity, or immunoactivity.

20K-hGH has been reported to have the same bioactivity as 22K-hGH in vivo (Lewis et al., 1978; Spencer et al., 1981; Closset et al., 1983), however, our results indicate that 20K-Met-hGH has poorer bioactivity than 22K hGH. Many unexpected factors may play a role in vivo. For example, Baumann et al., (1985) reported a slower metabolic clearance rate for 20K hGH than for 22K-hGH in rats. The characteristics of 20K-Met-hGH should therefore be determined with more appropriate in vivo models.

References


