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 (20 K 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 20 K 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 20 K hGH and methionyl 22 K 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 1×10^5 cells/well (24-well NUNC dish) using Fischer's medium supplemented with 10% horse serum, penicillin (50 U/ml), and streptomycin (50 μ g/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 μ g/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 $40\text{--}50 \times 10^6$ cells/ml. Aliquots containing 450 μ l of this cell suspension were placed in 12 $\times$ 75 mm glass tubes in

duplicate, to which was added 50 μ l 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 μ l of the same buffer. Aliquots, containing 450 μ l 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

Fig. 1. Effect of various concentration of samples in Nb₂ cell proliferation (after 96 hour incubation) Methionyl 20K 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).

Fig. 2. Inhibition of ^{125}I -P-hGH binding to IM-9 cells by increasing concentrations of unlabelled 20K-Met-hGH, 22K-Met-hGH, and pituitary hGH.

Fig. 3. Radioimmunoassay: The percent of ^{125}I -P-hGH bound to polyclonal antibodies produced with hGH (native), in the presence of unlabelled 20K-Met-hGH, 22K-Met-hGH, and pituitary hGH.

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}I -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 Nb₂ bioassay is based on the stimulation of the growth of Nb₂ rat lymphoma cell cultures by the lactogenic hormones. It has been shown that the Nb₂ cell line has prolactin receptors (Shiu *et al.*, 1983). Though the Nb₂ 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 Nb₂ bioassay however, does not reflect the somatotrophic, 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 methionate 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.

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