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20 kDa Human Growth Hormone (20K hGH) Stimulates Insulin-Like Growth Factor-I (IGF-I) Gene Expression at Lower Concentrations than 22K hGH in hGH Receptor-Expressing Ba/F3 Cells

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Abstract. Growth hormone (GH) secreted from the pituitary is essential for postnatal growth in animals. GH exerts its actions by a direct effect on target organs and by stimulating insulin-like growth factor I (IGF-I) production. In the human pituitary, there is a naturally occurring variant protein which has a molecular mass of 20 kDa (20K hGH) besides the major 22 kDa hGH (22K hGH), but the physiological actions of 20K hGH are still poorly understood. In this study we have examined its effects on the IGF-I mRNA expression in the pro B-cell line Ba/F3 cells stably expressing hGH receptor (Ba/F3-hGHR). Ba/F3-hGHR cells were incubated for 2 h with a series of various concentrations (10 pM~10 nM) of 20K or 22K hGH. The IGF-I mRNA expression in the Ba/F3-hGHR cells was detected by the RT-PCR method. IGF-I gene expression was increased by 20K and 22K hGH stimulation, but not by PRL or IL-3 in the Ba/F3-hGHR. And this effect was not observed in parental Ba/F3 cells. Lower concentrations of 20K hGH more strongly induced IGF-I gene expression than 22K-hGH. These results suggest that 20K and 22K hGH stimulate the IGF-I gene expression in the Ba/F3-hGHR through hGH receptors, and that the stronger effect of 20K hGH than that of 22K hGH in enhancing the IGF-I gene expression may be correlated with a 20K hGH specific receptor dimerization mechanism.

Key words: 20k hGH, 22K hGH, IGF-I expression, GH receptor

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GROWTH hormone (GH) exerts its growth promoting actions mainly by stimulating insulin-like growth factor I (IGF-I) production. In liver and skeletal muscle, IGF-I mRNA expression is known to be regulated by GH through the GH receptor (GHR) [1–3]. In the human pituitary gland and plasma, there is a naturally occurring variant GH protein whose molecular mass is 20 kDa (20K hGH) besides the major 22 kDa hGH (22K hGH) [4]. The 20K hGH lacks the amino acid residues 32 to 46 of 22K hGH and derives from the same gene (hGH-N) as 22K hGH by alternative mRNA splicing. 20K hGH has also been documented to possess full growth-promoting activity and somatomedin-generating potency as in 22K hGH via GHR *in vivo* [4, 5]. It also shows the same affinity for hGHR [6], although these two hGH isoforms have differences in clearance rates [7], and in the formation of GHR dimers and binding to GH binding protein [8]. But other physiological actions of 20K-hGH, especially the IGF-I mRNA expression which is one of the major effect of GH, are still poorly understood. In this study we have examined their effects on IGF-I mRNA expression in the pro B-cell line, Ba/F3 cells, stably expressing hGH receptor (Ba/F3-hGHR), and have found evidence that 20K hGH is more potent than 22K hGH in inducing IGF-I mRNA expression.

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Materials and Methods

Ba/F3 cells stably expressing hGH receptor (Ba/F3-hGHR) were kindly supplied by Dr. Honjo of Mitsui Chemical Inc (Chiba, Japan). Ba/F3-hGHR cells were maintained in a culture-selection medium (RPMI-1640 medium containing 1 mg/ml G418, 10% FBS, 50 μM 2-mercaptoethanol, 10 nM 22K-hGH and antibiotics) until grown to 1×10^6 cells/ml. The cells were incubated in the assay medium (RPMI-1640 medium with 0.5% FBS, 50 μM 2-mercaptoethanol and antibiotics) for 16 hours. And then Ba/F3-hGHR cells were incubated with a series of various concentrations (0 or 0.01 nM$\sim$ 10 nM) of 20K or 22K hGH for 2 h, and the cells were collected and washed with ice-cold PBS, and quickly frozen in liquid nitrogen until used. Recombinant 22K hGH was gift from Shikibo, Ltd. (Shiga, Japan) and recombinant 20K hGH was kindly supplied by Mitsui Chem. Inc.

Total RNA was extracted from cells by the guanidium isothiocyanate-phenol-chloroform method. IGF-IA, IB and β-actin mRNA expressions were detected by reverse transcription-polymerase chain reaction (RT-PCR)/Southern blot hybridization as described previously [9]. The primer pairs used for PCR of IGF-I cDNA can detect IGF-IA and IB at the same time and as different length of fragments (Fig. 1). Radioactivities of the Southern blot hybridization signals on the membranes were determined with a bioimage analyzer (BAS2000, Fuji Film, Japan). The data were analyzed for statistical significance by using the Macintosh SuperANOVA program and expressed as means$\pm$SE. The significance of differences between the values was analyzed by Scheffe's post hoc test, and $P<0.05$ was considered significant.

Results

The IGF-IA and IB mRNA expressions in the Ba/F3-hGHR cells were both detected by RT-PCR methods. The IGF-I gene expression levels were significantly increased by 2.37 to 10.81 fold and 1.47 to 10.72 fold with a series of concentrations of 20K and 22K hGH (Fig. 2), but not with PRL (data are not shown), but the levels of β-actin mRNA expressions were not changed by 20K, 22K hGH or PRL. Two GH isoforms had similar maximal effects on IGF-I gene expression, but lower concentrations (0.01 and 0.1 nM) of 20K hGH induced the IGF-I gene expression more strongly than 22K-hGH.

Discussion

20K hGH is naturally produced by alternative splicing of the pituitary hGH mRNA precursor and consequently lacks 15-amino acid (residues 32–46) of 22K hGH [4]. 20K hGH also exhibits full growth-promoting bioactivities and somatomedin-generating potency the same as the effects of 22K hGH mediating hGHR [4–6]. Moreover, previous studies reported that 20K hGH has the same binding affinity for hGHR and potency to induce the Spi2.1 gene expression through hGHR as 22K hGH [6]. But the

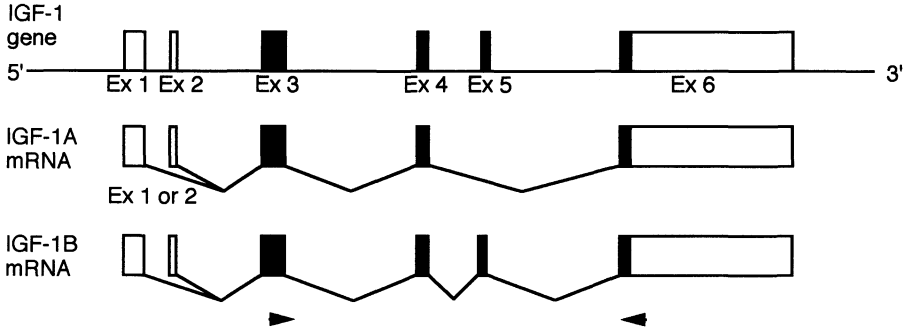

Fig. 1. Schematic presentation of the structural organization of the mouse IGF-I gene and two types of mRNA. IGF-IA and IB mRNAs are produced by alternative processing with a common primary transcript. The arrows indicate the locations of primers used for the PCR.

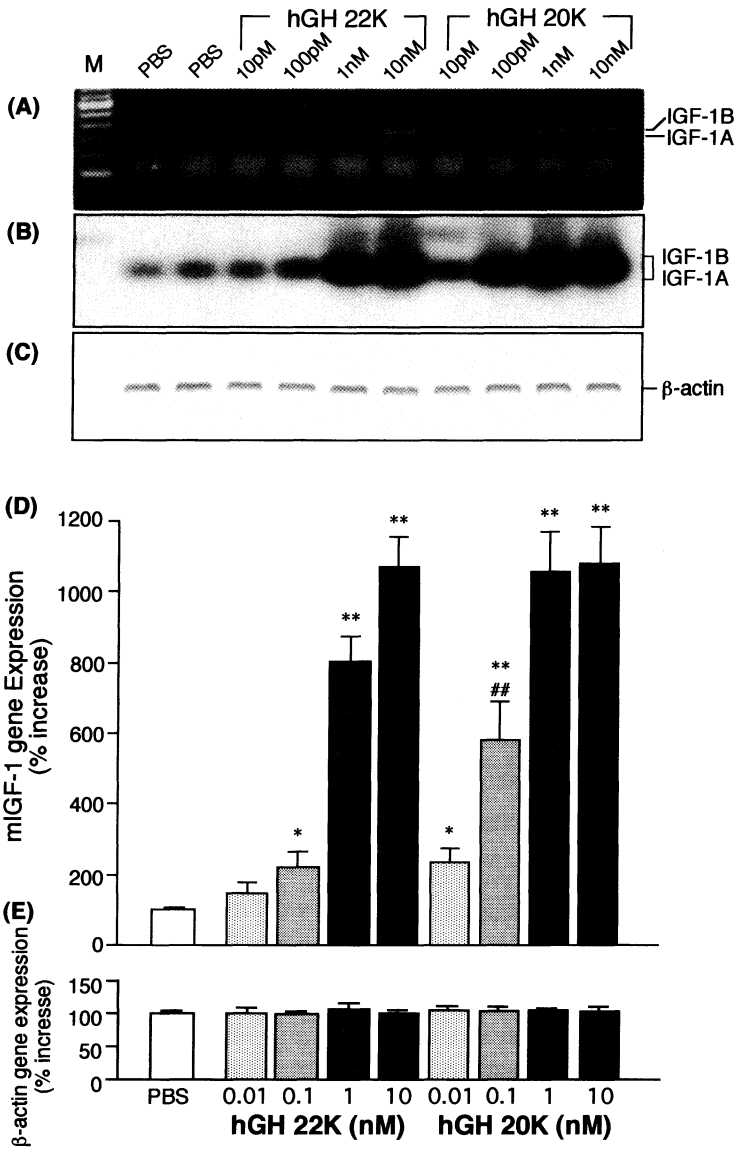

Fig. 2. Expression of IGF-I mRNAs which responded to a series of various doses of 20K and 22K hGH in Ba/F3-hGHR cells. The ethidium bromide staining and autoradiogram of amplified mouse IGF-IA and IB cDNAs are shown in A and B, respectively. C, Autoradiogram of mouse β-actin amplified cDNAs are shown. The radioactivities of the autoradiograms shown in B and C were determined with a BAS2000 bioimage analyzer (Fuji Film), and expressed as percent increases in the PBS control value in D and E in the lower panles. Each value is the mean±SME. *; $p<0.05$, **; $p<0.01$ *vs.* the PBS control.

effect on IGF-I production which is one of the most important actions of GH has not yet been evaluated. In the present study, we have shown that 20K hGH stimulates the IGF-I gene expression in a dose-dependent manner in Ba/F3-hGHR cells, as 22K hGH does. This effect was not observed with PRL or with hGHR-less cells (date not shown). These results suggest that 20K and 22K hGHs increase the IGF-I gene expression in the Ba/F3-hGHR cells, being mediated by hGHR. Moreover, we have found that lower concentrations of 20K hGH induced the IGF-I gene expression more effectively than 22K hGH. Recent studies by Wada *et al.* clearly showed that 20K hGH has a different receptor dimerization mechanism [8] and a different internalization rate [6] from those of 22K hGH. Another study has also

reported that comformational changes in hGHR after dimerization is required for hGH signaling [10]. We speculate that more potent activity in increasing the IGF-I gene expression with 20K hGH than with 22K hGH may be a result of the 20K hGH-specific receptor dimerization mechanism.

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