Regulation of human GH receptor gene transcription by 20 and 22 kDa GH in a human hepatoma cell line

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Abstract

The human GH gene is 1.7 kilobase pairs (kb) in length and is composed of five exons and four introns. This gene is expressed in the pituitary gland and encodes a 22 kDa protein. In addition to this predominant (75%) form, 5–10% of pituitary GH is present as a 20 kDa protein that has an amino acid (aa) sequence identical to the 22 kDa form except for a 15 aa internal deletion of residues 32–46 as a result of an alternative splicing event. Because it has been reported that non-22-kDa GH isoforms might be partly responsible for short stature and growth retardation in children, the aim of this study was to compare the impact of both 22 kDa and 20 kDa GH on GH receptor gene (GH receptor/GH binding protein (GHR/GHBP)) expression. Various concentrations of 20 kDa and 22 kDa GH (0, 2, 5, 12.5, 25, 50 and 150 ng/ml) were added to human hepatoma (HuH7) cells cultured in serum-free hormonally defined medium for 0, 1 and 2 h. Thereafter GHR/GHBP mRNA expression was measured by quantitative PCR. Addition of either 20 kDa or 22 kDa GH, at low or normal physiological concentrations (0, 2, 5, 12.5,

25 or 50 ng/ml) induced a dose-dependent increase in GHR/GHBP expression. However, a supraphysiological concentration of 20 kDa GH (150 ng/ml) resulted in a significantly lower ($P < 0.05$) downregulation of GHR/GHBP gene transcription compared with the downregulation achieved by this concentration of 22 kDa GH. This difference might be explained by a decreased ability to form a 1 : 1 complex with GHR and/or GHBP, which normally occurs at high concentrations of GH. Nuclear run-on experiments and GHBP determinations confirmed the changes in GHR/GHBP mRNA levels.

In conclusion, we report that both 20 kDa and 22 kDa GH, in low and normal physiological concentrations, have the same effect on regulation of GHR/GHBP gene transcription in a human hepatoma cell line. At a supra-physiological concentration of 150 ng/ml, however, 20 kDa GH has a less self-inhibitory effect than the 22 kDa form.

Journal of Endocrinology (2000) **165**, 313–320

Introduction

Human growth hormone (GH) consists of several structurally modified isoforms that have been detected in the pituitary gland and placenta and in the circulation (Baumann 1991, Lewis *et al.* 1994, Jansson *et al.* 1997). The major physiological component of pituitary gland secreted GH (about 75%) is a single chain polypeptide of 191 amino acids and a molecular mass of 22 kDa. At least four other monomeric GH isoforms, with molecular masses 27, 20, 17 and 5 kDa, have been detected in the circulation to date (Lewis *et al.* 1994). Furthermore, homo- and heterodimers are also secreted *in vivo*. Using the 22 kDa GH exclusion assay – an immunomagnetic extraction method for measurement of non-22-kDa GH isoforms in human blood (Boguszewski *et al.* 1996) – an average proportion of 8.1% (range 3.2–13.9%) of

non-22-kDa GH isoforms was reported in normal prepubertal children (Boguszewski *et al.* 1997). The 20 kDa GH comprises 5–10% of the pituitary derived GH and therefore represents the major non-22-kDa GH component.

The interaction between GH and its receptor is a sequential event, in which GH initially binds to one GH receptor (GHR) molecule through its site 1 to form an inactive 1 : 1 complex, followed by binding of site 2 to a second GHR molecule resulting in receptor homodimerization and activation (Cunningham *et al.* 1991).

As there is evidence that non-22-kDa GH isoforms might differ from the 22 kDa GH with respect to biological activity and GHR binding properties (Baumann & Shaw 1990, McCarter *et al.* 1990), the aim of this study was to evaluate the difference between 22 kDa and non-22-kDa GH in terms of their regulation of GHR/GH

binding protein (GHBP) gene expression. Furthermore, non-22-kDa isoforms may be weak agonists or even antagonists of the GHR binding, resulting in altered receptor homodimerization and activation depending on the relative affinities of binding sites 1 and 2 to the GHR (Goffin *et al.* 1996). Therefore, one could hypothesize that the mechanisms of some growth retardation in short children might be caused by GH isoforms other than the 22 kDa form. As the second major form is the 20 kDa GH, we studied the regulation of GHR/GHBP gene expression by these two naturally occurring isoforms of GH, namely the 22 kDa and 20 kDa GH.

Material and Methods

Cell culture and addition of recombinant 22 kDa and 20 kDa GH

HuH7 is a human hepatoma cell line that is reported to retain differentiated functions in culture (Nakabayashi *et al.* 1982) and allows functional studies of GHR/GHBP gene expression. HuH7 cells were maintained in monolayer culture as previously described (Mullis *et al.* 1991). When the monolayer reached approximately 70% confluency the medium was aspirated, the cells were washed twice with PBS pH 7.4 (Sigma Chemicals, St Louis, MO, USA) and serum-free hormonally defined medium was added. Briefly, serum-free hormonally defined medium contains, as previously reported, 0.4 mM ornithine, 2.25 µg/ml L-lactic acid, 500 ng/ml glucagon, 2.5×10^{-8} M selenium, 5×10^{-8} M hydrocortisone, 1×10^{-6} M ethanolamine, 2 ng/ml choleratoxin, 1 µg/ml insulin, 1 µg/ml transferrin, 25 ng/ml epidermal growth factor, but not GH, IGF-I or IGF-II (Barash *et al.* 1988). The Zn^{2+} concentration in the incubation solution was determined to be 10 µM by atomic absorption spectroscopy. After an overnight incubation, different concentrations of recombinant 22 kDa (Pharmacia & Upjohn, Stockholm, Sweden) and 20 kDa GH (Genentech Inc., South San Francisco, CA, USA) were added and the cultures were further incubated for 0, 1 and 2 h. These time intervals were chosen because the most striking differences in transcription rate were previously seen at these times (Mullis *et al.* 1991, 1995). In addition, maximum internalization of GH is reported after 2 h, whereas hormone accumulation in the nucleus was saturated after 1 h (Lobie *et al.* 1994). Each experiment was repeated four times.

The doses of GH used were 0, 2, 5, 12.5, 25, 50 and 150 ng/ml; 0 and 2 ng/ml represent low concentrations, 5–50 ng/ml are physiological concentrations and 150 ng/ml is a supraphysiological concentration of GH.

RNA extraction

RNA was extracted as previously described (Mullis *et al.* 1991) using the acid guanidium thiocyanate–phenol–

chloroform method (Chomczynski & Sacchi 1987). The RNA concentration was measured by absorbance at 260 nm using a double-beam Spectrophotometer (UV 150–02, Shimadzu Corporation, Tokyo, Japan).

Construction of the internal control and synthesis of internal control RNA

An internal control for quantitative PCR was constructed by inserting a 50 base pair (bp) fragment of the rat prolactin (PRL) receptor DNA into a portion of the GHR cDNA as previously described (Martini *et al.* 1995). Briefly, the subsequent chimeric plasmid of GHR was cleaved with EcoRV and EcoRI, and a 545 bp fragment was obtained. This fragment was subcloned into the SmaI–EcoRI sites of pBluescript SK+ (Stratagene Cloning System, San Diego, CA, USA). After digestion with SalI, the chimeric GHR construct served as a template for *in vitro* transcription by T7 RNA polymerase to generate internal cRNA. The cRNA was purified with phenol–chloroform extraction and subsequently by oligo (deoxythymidine) chromatography. The absolute number of cRNA molecules was calculated using spectrophotometric absorbance at 260 nm, the molecular weight of the cRNA molecule (216 600 g/mol) and Avogadro's number.

Oligonucleotide primers used for amplification

Oligonucleotide primers were purchased from Microsynth, Balgach, Switzerland. The forward primer was 5'-CCC TAT ATT GAC AAC ATC AGT TCC-3'; nucleotides 624–647 (exon 7 (Leung *et al.* 1987)) and the antisense primer was 5'-TTT CCT TCC TTG AGG AGA TCT GG-3' nucleotides 931–954 (exon 9 (Leung *et al.* 1987)).

cDNA synthesis and PCR amplification

Four micrograms total RNA and 2.0×10^6 molecules of internal control cRNA were reverse transcribed with 200 U Moloney murine leukaemia virus reverse transcriptase (RT-M-MLV; Gibco-Brl, Life Technologies, Basel, Switzerland) primed with 1 µg oligo (deoxythymidine)_{12–18} primer (Boehringer-Mannheim, Rotkreuz, Switzerland). The RT reaction was carried out in 20 µl (total volume) RT buffer (50 mM KCl, 2 mM Mg₂Cl and 20 mM Tris–HCl, pH 8.3), 1 mM of each deoxy-NTP, 1 mM dithiothreitol, and 20 U RNasin (Catalys, Promega, Wallisellen, Switzerland). Total RNA, cRNA and oligo (dT)_{12–18} were heated to 70 °C for 10 min and chilled on ice before addition of RT-M-MLV, RNasin, dNTP and RT buffer. The reaction was incubated for 60 min at 37 °C and subsequently chilled on ice. The PCR amplification was carried out using 8 µl each RT mixture in PCR buffer (50 mM KCl, 2 mM Mg₂Cl, and 20 mM Tris–HCl, pH 8.3), 200 µM deoxy-NTPs, 25 pmol

forward and reverse primers, 5 µl 50% formamide, 1×10^6 c.p.m. ^{32}P -end-labelled GHR sense primer, and 1.5 U Ampli-Taq-DNA (Perkin-Elmer, Rotkreuz, Switzerland) in a total volume of 50 µl. Sense primer was 5'-end-labelled with $[\gamma\text{-}^{32}\text{P}]$ ATP (>5000 Ci/mmol; Amersham, Zürich, Switzerland) using T4 polynucleotide kinase (Amersham). The amplification conditions were as follows: 24 cycles of denaturation for 45 s at 94 °C, 1.5 min annealing at 55 °C, and 1.5 min of extension at 72 °C, after the initial denaturation of 95 °C for 1.5 min. Amplification was completed with an additional extension step at 72 °C for 10 min. Twenty-four cycles of PCR, performed in an AMS Protocol thermal cycler (AMS, Lugano, Switzerland), amplified both GHR mRNA and cRNA in the linear range.

Analysis of the PCR-amplified cDNA product

PCR products (21 µl) were separated on a 2% Metaphor (FMC, BioProduct, Bio Concept, Allschwil, Switzerland) gel and stained with ethidium bromide. The bands corresponding to each specific PCR product were excised from the gels, and the amount of incorporated radioactivity was determined in a β -scintillation counter (MR-300 automatic liquid scintillation system, Kontron, Zeiss AG, Zürich, Switzerland). Radioactivity (c.p.m.) was plotted against the amount of template (cRNA or target molecule). Gel pieces of the negative control were excised at the position of the same size as that of each positive band. The radioactivity of the negative control values served as background.

Quantification of GHR/GHBP mRNA

The radioactivity (c.p.m.) recovered from each gel slice was plotted against either the amount of total RNA (ng) or cRNA (molecules). Previous studies have shown that the two curves are parallel and exponential for both cRNA and GHR/GHBP mRNA, suggesting that the quantitative PCR is equally efficient for both control and target RNA, without any competition that could interfere with reliable quantification (Martini *et al.* 1995).

Nuclear run-on transcription

Nuclear run-on assays were performed as previously described (Mullis *et al.* 1995). Briefly, nuclei were isolated from freshly washed pellets of GH-treated or untreated HuH7 cells and initiated transcripts were allowed to extend in the presence of $[\alpha\text{-}^{32}\text{P}]$ CTP (3000 Ci/mmol, New England Nuclear). Each run-on probe was hybridized with one of four identical filters dot-blotted with 10 µg GHR clone, β -tubulin clone pD β -1SK1 DNA and plasmid Bluescript SK+ DNA, as a negative control. After the DNA was cross-linked to the filters (Hybond-C,

Amersham), the filters were pre-hybridized overnight at 42 °C. Run-on probe (6×10^7 c.p.m.) synthesized from treated or untreated HuH7 nuclei was added, and the filters were hybridized at 42 °C for 72 h. The filters were washed, dried and autoradiographed. The strength of each autoradiographic signal was determined by liquid scintillation spectroscopy of excised filters pieces (Mullis *et al.* 1991, 1995). Briefly, the ratio of the counts corresponding to the GHR mRNA/ β -tubulin mRNA at time 2 h without any GH added was arbitrarily set as 1.0 unit, and served as baseline for measuring GHR/GHBP run-on transcription units. Importantly, β -tubulin mRNA levels in HuH7 cells did not change as a result of GH treatment and could, therefore, be used as a control for small variations in the quantity of RNA present in each sample analysed. The values obtained were consistent with values obtained by scanning densitometry (Bio-Rad Model 620 Video Densitometer, Bio-Rad Laboratories, Hemel Hempstead, Herts, UK) of the autoradiograph ($r=0.79$; $P<0.001$). In order to analyse whether the changes in the rate of GHR/GHBP gene transcription were dependent upon protein synthesis, a second set of experiments was performed with the addition of cycloheximide (10 µg/ml) to the culture medium 30 min before either GH.

Determination of GHBP

GHBP was measured as described previously (Holl *et al.* 1991, 1993), using a minor modification of the method originally published by Baumann *et al.* (1986). Briefly, 100 µl medium derived from the cell culture experiments was incubated with 30 000 c.p.m. ^{125}I -GH for 45 min at 37 °C, followed immediately by gel chromatography (Sephacryl S200, column 1.8×100 cm) at 4 °C using PBS. Radioactivity in the eluate (1 ml fractions) was quantified and the portion of ^{125}I -GH bound to GHBP, with an apparent molecular weight of 85 000, was calculated. The area of each peak was quantified using an objective computer program (PeakFit, Jandel Scientific, Erkrath, Germany). Chromatography on a long column separates an additional binding protein of low affinity/high capacity and thereby reduces non-specific binding (Baumann & Shaw 1990). A separate chromatography step to determine non-specific binding is therefore superfluous. GH was determined in each medium sample by immunoradiometric assay (IRMA) (Medgenix, Bruxelles, Belgium). The apparent binding of ^{125}I -GH was corrected for the GH concentration in the sample, based on a displacement curve using increasing amounts of unlabelled GH (Holl *et al.* 1991).

Statistical analysis

Statistical differences among experimental groups were determined by Student's unpaired two tailed *t*-test (Snedecor & Cochran 1980). Values of $P<0.05$ were

Table 1 Number of GHR/GHBP mRNA molecules in HuH7 cells determined by using quantitative PCR. Results are expressed as mean ± s.e.m. Each assay was performed in quadruplicate

		GHR/GHBP mRNA (× 10 ⁶ mol/mg total RNA)					
Incubation time (h):		0		1		2	
		<div><div></div><div>P</div><div></div></div>		<div><div></div><div>P</div><div></div></div>		<div><div></div><div>P</div><div></div></div>	
GH (22 kDa) (ng/ml)	GH (20 kDa) (ng/ml)						
0	P	0	2.76 ± 0.05 ns	ns	2.60 ± 0.05 ns	ns	2.49 ± 0.06 ns
		0	2.73 ± 0.04 ns	ns	2.59 ± 0.06 ns	ns	2.48 ± 0.07 ns
2	P	2	2.75 ± 0.03 ns	ns	2.77 ± 0.08 ns	<0.01	2.89 ± 0.07 ns
		2	2.73 ± 0.05 ns	ns	2.79 ± 0.08 ns	<0.01	2.91 ± 0.06 ns
5	P	5	2.74 ± 0.06 ns	<0.05	2.91 ± 0.1 ns	<0.01	3.22 ± 0.09 ns
		5	2.78 ± 0.05 ns	<0.05	2.96 ± 0.09 ns	<0.01	3.35 ± 0.11 ns
12.5	P	12.5	2.72 ± 0.07 ns	<0.01	3.12 ± 0.13 ns	<0.01	3.41 ± 0.15 ns
		12.5	2.70 ± 0.06 ns	<0.01	3.09 ± 0.10 ns	<0.05	3.30 ± 0.11 ns
25	P	25	2.76 ± 0.07 ns	<0.001	4.22 ± 0.09 ns	<0.01	4.46 ± 0.12 ns
		25	2.73 ± 0.05 ns	<0.001	4.14 ± 0.06 ns	<0.01	4.35 ± 0.08 ns
50	P	50	2.70 ± 0.04 ns	<0.001	5.28 ± 0.08 ns	<0.001	5.72 ± 0.12 ns
		50	2.78 ± 0.08 ns	<0.001	5.18 ± 0.1 ns	<0.001	5.82 ± 0.07 ns
150	P	150	2.63 ± 0.10 ns	<0.001	1.61 ± 0.07 <0.05	<0.001	1.27 ± 0.08 <0.01
		150	2.71 ± 0.09 ns	<0.001	2.04 ± 0.09 ns	<0.01	1.73 ± 0.1 ns

Figure 1 Effect of different concentrations of 20 kDa and 22 kDa GH, following 2 h of incubation, on transcription of GHR/GHBP gene in HuH7 cells analysed by run-on experiments. The autoradiographic signals were quantified by liquid scintillation counting of filter pieces as described in Methods. The ratio of signal obtained with GHR/GHBP target DNA to that obtained with β -tubulin target DNA was calculated for each set of culture conditions. This ratio was arbitrarily set at 1 unit for untreated HuH7 cells, and other values were adjusted accordingly and plotted. Values plotted are means of values obtained in four parallel experiments from four individual cultures.

in the quantitative PCR experiments was confirmed ($P < 0.005$; Fig. 1). The addition of cycloheximide did not change the transcription rates observed (Fig. 1). The alterations in transcription are therefore dependent on pre-existing factors and do not require protein synthesis.

Determination of GHBP concentrations in the culture medium

In order to define whether changes in expression might be reflected in protein concentrations after 20 kDa and 22 kDa GH incubations, GHBP concentrations were determined in the cell culture medium using the same experimental procedure as for the RNA quantification. The binding of ^{125}I -GH was presented as corrected percentage binding and values shown as mean $\pm$ S.E.M. As shown in Fig. 2, binding of ^{125}I -GH after correction for the amount of GH present in the culture medium increased significantly after the addition of 5 ng/ml GH or more, whereas 2 ng/ml GH added did not increase GHBP (Fig. 2). Importantly, there was no statistical difference in GHBP measured in the two experiments in which either 20 kDa or 22 kDa GH was used in low or normal physiological concentrations. At a supraphysiological concentration (150 ng/ml) there was a significant difference, the 20 kDa GH resulting in a smaller concentration of GHBP ($P < 0.05$) in the culture medium compared with 22 kDa GH.

Discussion

Human GH is produced as a single-chain, 191 amino acid 22 kDa protein. It is not glycosylated but contains two intramolecular disulphide bonds (Seeburg 1982, Hirt *et al.* 1987). Normally, about 75% of GH produced by the pituitary gland is of the mature 22 kDa form (Baumann 1991). Alternative splicing of the second intron, whereby the normal exon 3 splice-acceptor site is bypassed for a secondary site 15 codons downstream within exon 3, results in a deletion of amino acids 32–46, yielding a 20 kDa form, which normally accounts for 5–10% of pituitary GH (Cooke *et al.* 1988). In addition, Boguszewski *et al.* (1997) reported an increased proportion of non-22-kDa GH isoforms present in the circulation during spontaneous GH peaks in some prepubertal short children. As the second major form is the 20 kDa GH, the aim of our study was to analyse the regulation of GHR/GHBP gene expression by these two naturally occurring isoforms of GH, namely the 22 kDa and 20 kDa GH.

In a previous study McCarter *et al.* (1990) reported that the 20 kDa-GH variant did not bind to GHR in human liver obtained at autopsies 8–12 h *post mortem* and concluded that in humans the 20 kDa GH form plays little role in the somatogenic pathway of GH action. Our data, however, using HuH7 cells – a human hepatoma cell line that is reported to retain differentiated function in culture

Figure 2 Different concentrations of 20 kDa and 22 kDa GH were added to the culture medium as described in Methods. Thereafter, GHBP was measured after 2 h of incubation. Values plotted are means of values obtained in four parallel experiments from four individual cultures.

(Nakabayashi *et al.* 1982) – are in contrast with the findings of that study. We have shown similar effects of 20 kDa and 22 kDa GH at low (0 and 2 ng/ml) and normal (5, 12.5, 25 or 50 ng/ml) GH concentrations on the regulation of the GHR/GHBP gene expression, whereas a difference was observed in experiments in which a supraphysiological dose of 150 ng/ml was added to the culture medium. Regulation at the mRNA level was further studied and confirmed by run-on experiments and measurement of GHBP in the medium after the different incubation periods. In general, both 20 kDa and 22 kDa GH at a concentration of 5 ng/ml already resulted in a statistically significant increase in GHR/GHBP mRNA levels that was further upregulated by GH concentrations in the physiological range (up to 50 ng/ml). Supraphysiological concentrations of 150 ng/ml, however, downregulated the GHR/GHBP at the mRNA level, whereas the concentration of GHBP in the cell culture medium remained increased. This finding has been reported previously (Mullis *et al.* 1995). A possible explanation for the discrepancy between GHR/GHBP mRNA levels and those of GHBP might be that GHBP competes with the GHR for GH binding (Lim *et al.* 1990, Mannor *et al.* 1991) and may modulate the action of GH on target cells. It is therefore intuitively attractive to postulate that the cell system may protect itself from overstimulation by GH, as in rodents GH also induces GHBP but not GHR expression (Maiter *et al.* 1992).

Although similar regulation of GHR/GHBP gene transcription was observed at low and normal 22 kDa and

20 kDa GH concentrations, it is most important to stress that there was a discrepancy in GHR/GHBP mRNA levels between experiments in which high concentrations of 20 kDa or 22 kDa GH were added. The downregulation at the GHR/GHBP mRNA level was significantly smaller using 20 kDa GH than that observed after the addition of an equal dose of 22 kDa GH. These data were confirmed performing the run-on assays (Fig. 1). These findings are supported by results from studies looking at the complex formation of 20 kDa- and 22 kDa GH with cell surface GHR (Wada *et al.* 1998). They reported that the affinity for 1 : 1 complex formation of 20 kDa GH and GHR is reduced to 1 : 10, whereas for 1 : 2 complex formation it is 10 times higher compared with the complex formation of 22 kDa GH with GHR (Uchida *et al.* 1998). Therefore, in view of the sequential dimerization model it has been shown that the 20 kDa GH has difficulties in generating a 1 : 1 complex with GHR and, even more importantly, with GHBP, even though it can generate a 1 : 2 (GH : GHR) complex to the same or better extent as the 22 kDa GH. These findings suggest that the 15 amino acid deletion in the 20 kDa GH form permits a conformational change allowing increased 1 : 2 complex formation (Wada *et al.* 1998). Regarding the interaction of 22 kDa GH with the GHR, considerable information has been accumulated (Baumann & Shaw 1990, McCarter *et al.* 1990, Baumann *et al.* 1994, Postel-Vinay & Finidori 1995). At low and normal physiological GH concentrations, 22 kDa GH is bound to the GHR as a single 1 : 2 (GH : GHR) complex. However, whenever the

ratio GH : GHR increases, the formation of a 1 : 1 (GH : GHR) complex is favoured, rendering the complex inactive in terms of signal transduction. Therefore, the reported problems in the formation of functionally inactive 1 : 1 (20 kDa GH : GHR) complexes may explain why 20 kDa GH in high concentrations has a less self-inhibitory effect, whereas at low and normal physiological concentrations – which result mainly in the formation of 1 : 2 (20-kDa GH : GHR or 22 kDa GH : GHR) complexes 20 kDa GH has the same bioactivity as 22 kDa GH. This hypothesis is supported by previous binding studies, stoichiometric analyses (Baumann *et al.* 1994, Uchida *et al.* 1998, Wada *et al.* 1998) and by our measurement of GHR/GHBP mRNA levels.

Another possible mechanism explaining the discrepancy between GHR/GHBP mRNA levels and GHBP concentrations when cells are treated with supraphysiological concentrations of 20 kDa and 22 kDa GH could be that the two GH-isoforms have a different efficiency in GHBP glycosylation and/or in cleavage of the GHR. Furthermore, as GHR is expressed in a tissue-specific manner and multiple mRNA variants (V) such as V1–V8 have been detected in human tissues (Pekhletsky *et al.* 1992), the liver specific promoter and/or promoters of GHR might be differently regulated on different GH concentrations (Zou *et al.* 1997, Schwarztbauer & Menon 1998).

The physiological and clinical relevance of different effects of supraphysiological concentrations of 20 kDa and 22 kDa GH isoforms on regulation of GHR/GHBP gene transcription might be in some clinical situations in which a high proportion of 20 kDa GH in combination with increased 22 kDa has been found, for example in anorexia nervosa and active acromegaly (Tsushima *et al.* 1999). However, further studies are needed to elucidate these *in vivo* disorders. In addition, there might be some interest in future on the impact of these high GH concentrations in specific situations in which high levels of GH are desired; for example, we should not forget about the abuse of GH in sports.

In conclusion, we report that 20 kDa and 22 kDa GH in low and normal physiological concentration have the same effect on regulation of GHR/GHBP gene transcription in a human hepatoma cell line. In a supraphysiological concentration of 150 ng/ml, however, 20 kDa GH has a less self-inhibitory effect than the 22 kDa form, which might be explained by a decreased ability to form a 1 : 1 complex with either GHR and/or GHBP that normally occurs in high concentrations of GH.

Acknowledgements

The GH-receptor cDNA clone pghr 501.●1 was a generous gift from Dr W Wood from Genentech Inc., San Francisco, CA, USA and the β -tubulin cDNA clone D β -1 was from Dr N J Cowan, University Medical

Center, New York. We would like to thank Professor Iain C A F Robinson, National Institute for Medical Research, Mill Hill, London, UK for providing the 20 kDa GH and for his kind help and valuable advice while reviewing the manuscript. The study was supported by The Swiss National Science Foundation (3200–53714-98). Furthermore, we would like to thank Pharmacia & Upjohn, Stockholm, Sweden for the recombinant GH and Novo-Nordisk, Knsnacht, Switzerland and Pharmacia & Upjohn, Dbendorf, Switzerland for financial support.

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Received 6 September 1999

Accepted 20 January 2000