Growth hormone (GH) receptors, binding proteins and IGF-I concentrations in the serum of transgenic mice expressing bovine GH agonist or antagonist

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Abstract

We have examined the regulation of hepatic growth hormone receptors (GH-R) and serum GH binding proteins (GHBP) in transgenic mice expressing an antagonist of bovine growth hormone (bGH), G119K-bGH, and consequently exhibiting a growth suppressed dwarf phenotype. Specific GHBP could be measured in transgenic dwarf mouse serum only by immunological methods (RIA), because these mice have a very high concentration of mutated bGH in circulation ($>1 \mu\text{g/ml}$) and, therefore, almost all GHBP is bound to G119K-bGH and cannot be quantitated in binding assays. The concentrations of GHBP were $0.6 \pm 0.4 \text{ nM}$ and $1.7 \pm 0.4 \text{ nM}$ for normal and dwarf mice respectively. The concentrations of free GHBP in normal mice and in transgenic mice expressing wild-type GH can be calculated using chromatographic techniques as the dissociation constant (K_d) and the ratio of bound ^{125}I -GH to free ^{125}I -GH in the serum ($[\text{GHBP}]_{\text{free}} = B/F \cdot K_d$). In agreement with the assumption that GHBP reflects GH-R status, liver uptake of injected labeled bGH was greatly reduced in transgenic dwarfs in comparison with normal mice or with transgenic mice expressing wild-type bGH (liver/blood ratio of 0.48 ± 0.21 , 2.7 ± 0.2 , and 1.3 ± 0.3 respectively) indicating that the high concentration of the mutated bGH

(G119K-bGH) prevents labeled bGH uptake, as was expected from the dwarf phenotype. ^{125}I -bGH taken up by the liver of transgenic dwarf mice was found in a smaller molecular species than in normal mice, compatible with the presence of 1:1 [(GH-R):GH] complexes instead of the 2:1 [(GH-R)₂:GH] or 2:2 [(GHBP)₂:(GH)₂] complexes found in normal mice.

The concentration of IGF-I, the principal mediator of GH activity, in the G119K-bGH transgenic mice was correlated with the concentration of free GHBP. This allowed us to use free GHBP concentration as a marker of the effects of the active endogenous hormone (mGH) on liver receptors in the presence of different concentrations of the antagonist of GH.

The levels of GHBP in serum, as well as the concentration of GH-R in liver microsomes from mice expressing the bGH antagonist, are up-regulated by the high concentration of G119K-bGH (85%), but significantly less so than that which could be expected for the same concentration of native GH (220–275%). This up-regulation suggests that the G119K-bGH antagonist is internalized and induces synthesis of the receptor and of the binding protein.

Journal of Endocrinology (1998) **158**, 53–59

Introduction

Bovine growth hormone (bGH) analogs produced by site-directed mutagenesis, G119R-bGH and G119K-bGH, competitively antagonize the action of endogenous GH both *in vivo* and *in vitro* (Chen *et al.* 1991a,b, Chen *et al.* 1995, Xu *et al.* 1995). Since this inhibitory action and its physiological sequelae have not been completely characterized, we have examined the effects of this analog on the regulation and characteristics of hepatic GH receptor (GH-R) and serum GH binding protein (GHBP), and on the concentration of insulin-like growth

factor-I (IGF-I) in transgenic dwarf mice expressing G119K-bGH antagonist. Since overexpression of wild-type bGH in transgenic mice is associated with significant up-regulation of GH-R and GHBP (Aguilar *et al.* 1992, Sotelo *et al.* 1995), we were particularly interested in the effects of bGH antagonist on the levels of liver membrane GH-R and serum GHBP.

Since the levels of GHBP are closely related to GH-R levels (Amit *et al.* 1992, Cramer *et al.* 1992b, Baumann *et al.* 1994a, Ilkbahar *et al.* 1995), we have studied GHBP to obtain information on the competitive inhibition of GH binding by G119K-bGH *in vivo*. This was accomplished

by estimating the concentration of free, unbound GHBP in normal mouse serum and in the presence of different concentrations of the antagonist. The concentration of free GHBP is an important parameter for monitoring the action of GH on the liver and for assessing the inhibitory effects of the antagonist on the actions of GH, such as stimulation of IGF-I levels. This information is pertinent to the evaluation of the potential usefulness of GH antagonists in the treatment of pathologies with supranormal levels of GH.

In this report, we also present evidence for the competitive inhibitory action of the G119K-bGH analog on the hepatic uptake of ^{125}I -bGH *in vivo*. Since the mechanism of GH action is thought to involve formation of 2:1 [(GH-R)₂:GH] complexes on the membrane surface (Baumann *et al.* 1994b) and G119K bGH appears to form only 1:1 [(GH-R):GH] complexes as originally proposed by Chen *et al.* (1991a,b), we discuss the implications of this finding for interpretation of the results obtained in transgenic mice overexpressing bGH antagonist.

Materials and Methods

Animals

Transgenic mice overexpressing the G119K-bGH gene linked to the promoter of mouse metallothionein-1 (MT) used in this work were derived from animals described previously (Chen *et al.* 1991a,b). This line of transgenic mice was maintained by crossing transgenic mice with B6SJL F1/J nontransgenic individuals (Chen *et al.* 1991a,b, Chen *et al.* 1995). PEPCK-bGH transgenic mice were derived from males (kindly provided by Drs T E Wagner and J S Yun, Ohio University, Athens, OH, USA) carrying one (PEPCK-bGH-1) or five (PEPCK-bGH-5) copies of the wild-type bGH gene under the control of the regulatory sequences from the rat phosphoenolpyruvate carboxykinase gene. Transgenic animals from these lines differ in the level of bGH expression (Steger *et al.* 1994).

Female mice were housed 3–5 per cage in a room with controlled light (12 h light/day) and temperature ($22 \pm 2^\circ\text{C}$). The animals had free access to food (Lab Diet, Formulab 5008, containing a minimum of 23% protein, 6.5% fat, and a maximum of 4% fiber; PMI Feeds, Inc., St Louis, MO, USA) and tap water.

Hormones

Bovine GH was prepared in our laboratory (Turyn & Dellacha 1978) and human and mouse GH were obtained through the National Hormone and Pituitary Program, NIDDK, NIH, Bethesda, MD, USA.

Radioiodination of hormones

Human, bovine and mouse GH were radiolabeled using limiting amounts of Chloramine T, as previously described

(Aguilar *et al.* 1992). Specific activity ranged from 70 to 100 $\mu\text{Ci}/\mu\text{g}$.

Radioreceptor and radioimmunoassays

Bovine (b) and mouse (m) GH levels were measured as described previously (Sotelo *et al.* 1993). Mutated bovine growth hormone (G119K-bGH) was determined as bGH, using polyclonal antibodies against bGH as described by Chen *et al.* (1991a,b). The radioreceptor assays (RRA) for mGH, bGH, and G119K-bGH were performed as previously described (Sotelo *et al.* 1995).

Serum IGF-I concentrations were measured, after extraction of its binding proteins, by RIA using a commercial kit for rat IGF-I from Diagnostic Systems Laboratories, Webster, TX, USA.

In vivo ^{125}I -bGH uptake

Determinations of the specific liver uptake and the molecular weight (MW) of radioactive complexes present in the liver were performed as previously described (Turyn & Bartke 1993, Turyn *et al.* 1997).

Microsomes binding assay

The ^{125}I -bGH binding assay was carried out as previously described (Aguilar *et al.* 1992) using liver microsomes obtained according to Borst & Sayare (1982), with an additional washing step using 50 mM ethanolic acid buffer (pH 5.0) to remove bound GH. Four to six livers were pooled for each membrane preparation.

GHBP assays

Chromatographic assays for GHBP were performed as previously described (Sotelo *et al.* 1995). The RIA for GHBP was performed as described (Cramer *et al.* 1992a) except that the 28 amino acid synthetic peptide of mouse GHBP C-terminus coupled to keyhole limpet hemocyanin (KLH), used in order to obtain antibodies against the GHBP, was from TANA Laboratories, Houston, TX, USA. This peptide was also used for the standard curve (Smith *et al.* 1989, Cramer *et al.* 1992b, Sotelo *et al.* 1995).

Processing data

The amounts of bound and free GHBP were calculated as B/F ratio $\times$ concentration of free GH (determined by RRA), and B/F ratio $\times K_d$, where K_d , B and F represent the dissociation constant, bound and free hormone concentrations respectively (Hochberg *et al.* 1993, Sotelo *et al.* 1995). Total GHBP was calculated by summation of bound and free GHBP values. The non-linear regression curve-fitting program Ligand (Munson & Rodbard 1980)

Table 1 Body weight, serum IGF-I and serum GH concentrations in normal and transgenic mice. GH concentrations in normal and transgenic mice (PEPCK-bGH-1 and -5, and G119K-bGH) were determined by specific RIA for mGH (normal mice) and bGH (transgenic mice), and by RRA for total, active GH (for experimental details see Materials and Methods). Serum IGF-I concentrations were determined by specific RIA. The data are means $\pm$ S.D. for the indicated number of animals (in parentheses)

	Body weight (g)	GH (ng/ml)		IGF-I (ng/ml)
		RRA	RIA	
Animals				
Normal	26 $\pm$ 0.6 (22)	17 $\pm$ 3 (9)	19 $\pm$ 4.6 ^a (8)	250 $\pm$ 8 (10)
PEPCK-bGH-5	48 $\pm$ 1.3 ^b (5)	301 $\pm$ 29 ^b (5)	466 $\pm$ 19 ^{b,c} (5)	508 $\pm$ 53 (3)
PEPCK-bGH-1	51 $\pm$ 2.2 ^b (5)	879 $\pm$ 8.5 ^b (5)	990 $\pm$ 121 ^{b,c} (5)	552 $\pm$ 8 ^b (5)
G119K-bGH	18 $\pm$ 0.6 ^b (23)	1850 $\pm$ 302 ^b (14)	2013 $\pm$ 90 ^{b,c} (19)	91 $\pm$ 5 ^b (13)

^aNot significantly different compared with RRA; ^b $P < 0.001$ compared with normal mice values; ^c $P < 0.001$ compared with RRA.

was used to analyze competition studies. The Scatchard analyses (Scatchard 1949) of competitive curves were used to derive estimates of the apparent dissociation constants (K_d) and binding site capacities (B_{max}). Data are presented as means $\pm$ S.E.M.; statistical differences between groups were determined using Student's paired or unpaired *t*-tests. When multiple comparisons were performed, the data were analyzed by ANOVA.

Chemicals

Ovine prolactin (oPRL) was from Sigma Chemical Co. (St Louis, MO, USA); keyhole limpet hemocyanin (KLH) was from Calbiochem Behring (La Jolla, CA, USA) and Na¹²⁵I was purchased from Dupont (Boston, MA, USA). All other chemicals were of reagent grade.

Results

Data on body weights, concentrations of GH measured by both RIA and RRA, and concentrations of IGF-I for the animals used in the present study are given in Table 1. It is important to emphasize that RRA measures available GH while RIA measures total GH concentration (Hochberg *et al.* 1993). The ratio of GH concentration measured by RRA to that measured by RIA ranged between 0.6 and 0.9 (Table 1). Serum binding of ¹²⁵I-bGH and/or ¹²⁵I-hGH was analyzed on 1.5 $\times$ 11.0 cm Sephacryl S200 HR columns, in order to obtain quantitative results and minimize dissociation of GH-GHBP complexes during chromatography (which was less than 8% after 20 min, the mean retention time of the GH-[GHBP] complexes). Two peaks of radioactivity were detected as expected from our previous studies (Sotelo *et al.* 1995). The first peak accounts for ¹²⁵I-GH bound to GHBP, and the second peak represents free ¹²⁵I-GH (Fig. 1A). Figure 1 shows representative elution profiles of ¹²⁵I-hGH incubated alone or together with an excess of unlabeled hGH with serum from normal or

transgenic mice. Matched comparisons between normal, transgenic PEPCK-bGH-1, transgenic PEPCK-bGH-5 and transgenic dwarf mice serum samples revealed major differences in binding of ¹²⁵I-GH to serum GHBP, expressed as a percentage (%) of total ¹²⁵I-GH added (Table 2). For G119K-bGH transgenic dwarf mice, labeled GH specifically bound to serum binding protein(s) did not differ from the values of non-specific binding in almost all of the samples measured (Table 2).

The dissociation constant of bGH binding to normal mouse serum calculated by Scatchard analysis of data was 6.3 ± 1.7 nM ($n=3$). The concentrations of GHBP determined by the chromatographic method and by the specific RIA for mouse GHBP are shown in Table 3.

Table 2 also includes the values of specific ¹²⁵I-bGH binding to liver microsomes obtained from normal and transgenic mice used in this study. Labeled bGH was used in order to determine somatogenic binding sites because bGH cannot be bound by the mouse prolactin receptor (Amit *et al.* 1992). In G119K-bGH transgenic mice, GH binding to the liver microsomes was increased by 85% over the values obtained for normal (non-transgenic) mice, while a 220–275% increase was obtained in microsomes from livers of transgenic mice overexpressing wild-type bGH (Table 2).

Table 4 shows the *in vivo* liver uptake measured 30 min after injecting ¹²⁵I-bGH either alone or together with an excess of unlabeled bGH, in normal and transgenic mice. The radioactivity taken up by the liver of normal mice and transgenic dwarf mice expressing G119K-bGH was analyzed on a Sepharose CL-6B column and is shown in Fig. 2.

In transgenic dwarf mouse plasma, the concentration of IGF-I corresponds to $36.7 \pm 7.4\%$ of the values measured in normal mice, while in transgenic PEPCK-bGH-1 mice expressing high concentrations of native bGH, plasma concentrations of IGF-I were increased $121 \pm 18.5\%$ over non-transgenics (Table 1).

Figure 1 Representative gel filtration profiles of mouse serum (10 µl) from non-transgenic control (normal) mice (A), transgenic PEPCK-bGH-5 mice (B), transgenic PEPCK-bGH-1 mice (C) and transgenic G119K-bGH dwarf mice (D), incubated with ¹²⁵I-hGH in the absence (○) or presence (●) of 1 µg unlabeled hGH, chromatographed on Sephacryl S200 HR 1.5 × 11 cm columns. The arrows indicate the elution volumes of Dextran Blue (V_o) and ¹²⁵I-hGH (GH). The results are representative of the number of experiments indicated in Table 2.

Table 2 Specific binding of ¹²⁵I-bGH to serum and to liver microsomes and of ¹²⁵I-hGH to the serum of normal and transgenic female mice. Data are means ± S.D. of the number of animals indicated in parentheses

	¹²⁵ I-bGH bound		¹²⁵ I-hGH bound
	Liver microsomes (µg/mg)	Serum (% specifically bound)	Serum (% specifically bound)
Animals			
Normal	3.82 ± 0.22 (6)	31.3 ± 3.6 (4)	35.8 ± 4.0 (11)
PEPCK-bGH-5	12.23 ± 1.25 ^b (6)	16.9 ± 2.6 ^b (3)	15.6 ± 3.8 ^b (5)
PEPCK-bGH-1	14.33 ± 0.88 ^b (6)	7.0 ± 2.3 ^b (3)	5.2 ± 1.2 ^b (5)
G119K-bGH	7.08 ± 0.99 ^b (6)	0.8 ± 1.1 ^{a,b} (7)	1.1 ± 1.0 ^{a,b} (18)

^aNot significantly different from zero; ^bP<0.001 compared with normal.

Discussion

Numerous findings support the idea that circulating GHBP levels reflect GH-R status (Baumann *et al.* 1994a). However, the regulation of GHBP and/or GH-R is not

fully elucidated, and some controversial findings were reported in former animal studies (Turyn & Dellacha 1978, Amit *et al.* 1992). We have demonstrated up-regulation of GH-R in liver membranes and GHBP in serum in transgenic mice expressing different levels

Table 3 Determination of GHBP concentration by a chromatographic method and by RIA. The data are the means $\pm$ S.D. of the number of animals indicated in parentheses

Animals	Chromatographic method (nM)	RIA (nM) ^a
Normal	1.1 $\pm$ 0.2 (11)	0.6 $\pm$ 0.4 ^c (6)
PEPCK-bGH-5	3.8 $\pm$ 0.8 ^b (5)	3.9 $\pm$ 1.2 ^b (6)
PEPCK-bGH-1	3.3 $\pm$ 1.5 ^b (5)	4.5 $\pm$ 2.0 ^b (6)
G119K-bGH	ND (18)	1.7 $\pm$ 0.4 ^b (6)

ND, non-detectable, below detection limit.

^aA synthetic peptide of 28 amino acids was used as reference (see Materials and Methods).

^b $P < 0.001$ compared with normal; ^c $0.01 < P < 0.02$ compared with the chromatographic method.

Table 4 *In vivo* uptake of ¹²⁵I-bGH in normal and transgenic mice. *In vivo* uptake 30 min after injection (i.v.) of ¹²⁵I-bGH alone or together with an excess of unlabeled bGH (300 μ g/50 g bodyweight) into normal or transgenic mice, expressed as L/B ratio. L/B ratio represents the ratio of radioactivity per gram of liver over the radioactivity per gram of blood. The data are the means $\pm$ S.D. of five determinations

Animals	¹²⁵ I-bGH	¹²⁵ I-bGH+bGH
Normal	2.7 $\pm$ 0.2	0.50 $\pm$ 0.20 ^b
PEPCK-bGH-5	1.3 $\pm$ 0.2 ^a	0.35 $\pm$ 0.15 ^b
PEPCK-bGH-1	1.0 $\pm$ 0.3 ^a	0.44 $\pm$ 0.18 ^c
G119K-bGH	0.48 $\pm$ 0.21 ^a	0.42 $\pm$ 0.18 ^d

^a $P < 0.001$ compared with normal; ^b $P < 0.001$ compared with injected

¹²⁵I-bGH alone for the same strain of mice; ^c $0.001 < P < 0.01$ compared with injected ¹²⁵I-bGH alone; ^dnot significantly different compared with injected ¹²⁵I-bGH alone.

of heterologous GH (Aguilar *et al.* 1992, Sotelo *et al.* 1995).

In the present work we have examined the levels of GHBP in transgenic mice expressing a bGH analog (G119K-bGH) that antagonizes the action of wild-type GH, and compared the results with those obtained in normal mice and two lines of transgenic mice expressing different amounts of wild-type bGH (Sotelo *et al.* 1995).

We have used chromatographic and immunological techniques to measure free and total GHBP respectively (Hochberg *et al.* 1993, Sotelo *et al.* 1995), since we have previously found a good correlation between free GHBP measurements using this technique and results of a specific GHBP RIA for normal and transgenic mice overexpressing wild-type GH (Sotelo *et al.* 1995). In normal mice, an important fraction of GHBP was bound to GH, in agreement with previous observations (Sotelo *et al.* 1995). In PEPCK-bGH-5 and PEPCK-bGH-1 mice, the percentage of free GHBP was reduced in direct proportion to available GH plasma levels as determined by RRA (Hochberg *et al.* 1993). In transgenic G119K-bGH dwarf mice, all detectable GHBP is bound to the GH

Figure 2 Sepharose CL-6B elution profiles of ¹²⁵I-bGH taken up *in vivo* by the liver of non-transgenic (normal) mice (A) and G119K-bGH transgenic (B) mice. The livers from female mice that had previously been injected i.v. with ¹²⁵I-bGH alone (○) or with 300 μ g/50 g body weight unlabeled bGH (●) were homogenized in Tris-HCl buffer (25 mM), pH 7.4, containing 1% Triton X-100, and centrifuged at 100 000 *g* for one hour. An aliquot of the supernatant (0.8–1.5 ml) was chromatographed on a Sepharose CL-6B column (see Materials and Methods). The arrows indicate the elution volume of Blue Dextran (V_0), ¹²⁵I-bGH (GH) and Na¹²⁵I (V_t). II and III represent the elution volumes of the [¹²⁵I-bGH-GHR] complexes. The results are a representative example from four experiments.

antagonist in agreement with the observation that no significant binding of ¹²⁵I-GH was detected (Fig. 1D and Table 2). Previously, we had observed an increase in GHBP levels in transgenic mice expressing the wild-type bGH gene, in which plasma GH levels were elevated up to 40-fold, compared with non-transgenic mice (Sotelo *et al.* 1995). These animals provide excellent models to estimate the increase and saturation of GH-R and GHBP due to the elevated concentration of available GH, and to compare them with the results obtained for the mice expressing the bGH antagonist. This allows us to

distinguish between the effects of GH agonists and antagonists in mice. As is shown in Table 3, the levels of GHBP were increased only 180% over control values in transgenic dwarf mice overexpressing G119K-bGH, while an increase of 550–650% over control was found in both lines of transgenic mice expressing different amounts of the wild-type bGH. This is in spite of the G119K-bGH concentration being between 2 and 9 times higher in the transgenic dwarf mice than the bGH levels in the other transgenic lines (Table 1). A similar conclusion could be made from the values of ^{125}I -bGH bound to liver microsomes from normal and transgenic mice (Table 2). The up-regulation of both GHBP and GH-R by the bGH antagonist, although smaller in magnitude than that in transgenic mice overexpressing wild-type bGH, suggests that G119K-bGH is internalized in order to induce the synthesis of the receptor and the binding proteins, as was proposed by Harding *et al.* (1996) and Chen *et al.* (1991a,b). Since no binding of ^{125}I -GH was found in the serum of transgenic dwarf mice, almost all of the GHBP is bound to mutated G119K-bGH (Fig. 1 and Table 2), clearly demonstrating that the GH antagonist could saturate the GHBP *in vivo*. Considering that the affinity constants for GH-R and GHBP are comparable (Hochberg *et al.* 1993), GH receptors should also be saturated in these mice. This conclusion is further supported by the fact that the uptake of labeled bGH in these transgenic mice is essentially reduced to values of non-specific uptake (Table 4; Turyn & Dellacha 1978, Turyn & Bartke 1993, Turyn *et al.* 1997). From these results, it is possible to calculate the concentration of GH antagonist needed to reduce the amount of endogenous mGH bound to the receptor and consequently to reduce its biological activity. A concentration over 2000 ng/ml is necessary to prevent completely the binding of active GH (data not shown). These data represent a mean competition constant ($\text{ED}_{50\%}$) of 8 and 10.6 nM (180 and 240 ng/ml) for bovine and human GH respectively, consistent with a K_d of 6.3 nM obtained for normal mice. The value of K_d for normal mice is in agreement with previous data for normal and transgenic mice, including transgenic dwarf mice (Chen *et al.* 1991a,b, Baumann *et al.* 1994a, Chen *et al.* 1995, Sotelo *et al.* 1995). We calculated the $\text{ED}_{50\%}$ values, since the concentration of total GHBP as well as that of GH-R are different in the four types of mice studied (Table 3). Lower concentrations of GH antagonist allow more of the available mGH to bind, and thus to exhibit biological activity. Consequently, an extremely high concentration of the antagonist (up to 2000 ng/ml) should be considered for potential use in medical therapy. The result represents a balance between the up-regulation of membrane GH-R, that produces an increase in GH-dependent activities, and the inhibition of mGH binding, that leads to suppression of GH-dependent activities. One of the principal actions of GH is to stimulate IGF-I production, primarily in the liver, following active GH binding to its

receptor(s) (Zapf & Froesch 1986). For this reason, the determination of IGF-I concentration should correspond to the inhibition of mGH binding to liver receptors in the presence of the GH antagonist.

It is interesting to note that some transgenic dwarf mice express GH concentrations which are much lower than the average (2013 ng/ml), as was previously reported (Chen *et al.* 1991a,b, Chen *et al.* 1995). These smaller concentrations of GH antagonist generally produce lesser or no reduction of body weight (Chen *et al.* 1991a,b, Chen *et al.* 1995). This was seen in 3 individuals among the 27 transgenic dwarf mice used in the present study. In these three mice, the concentrations of bGH antagonist were 399, 590 and 850 ng/ml, and the percentage of ^{125}I -hGH bound to serum GHBP was substantially higher (15.3%, 14.8%, and 7.0% respectively) than the values obtained in the remaining 23 transgenic dwarf mice: $0.8 \pm 1.1\%$ for ^{125}I -bGH and $1.1 \pm 1.0\%$ for ^{125}I -hGH (Table 2). These values are in agreement with a linear relationship found for the percentage of ^{125}I -hGH bound to serum from normal mice and transgenic mice expressing wild-type bGH or G119K-bGH and the log of GH concentration. This agreement can be extended to IGF-I levels, since maximal inhibition of IGF-I production was seen in G119K-bGH mice expressing high concentrations of the analog, while a less severe inhibition of IGF-I production was seen in three transgenic dwarf mice with low plasma levels of G119K-bGH (data not shown). These observations provide the basis for using the concentration of free GHBP as a tool to assess the B/F ratio of liver GH-R and to relate it to its activity.

The MW of the GH:(GH-R) complexes obtained for normal mice is in accordance with previous data for normal and transgenic mice overexpressing wild-type GH, and is compatible with the structure of 1:2 [GH:(GH-R)₂] or 2:2 [GH₂:(GH-R)₂] complexes, with a Stokes radius of 64 Å (Ciccía-Torres *et al.* 1983, Turyn & Bartke 1993, Harding *et al.* 1996, Turyn *et al.* 1997). The complexes obtained *in vivo* for transgenic mice overexpressing the bGH antagonist had a significantly smaller MW (peak III in Fig. 2B) than those obtained for normal mice (peak II of Fig. 2A), suggesting that the antagonist is unable to generate the dimerization of the GH-R, as was previously reported (Chen *et al.* 1991a,b, Chen *et al.* 1995, Harding *et al.* 1996). The GH-R bound to ^{125}I -bGH is unable to dimerize, since almost all of the receptors are bound to the G119K-bGH analog, and no free, accessible GH-R molecules are available (Fig. 2 and Tables 2 and 3).

In summary, we have demonstrated the presence of GHBP in the plasma of transgenic G119K-bGH dwarf mice and provided evidence that the concentration of bGH analog in the circulation of most of these animals is sufficient to completely saturate circulating GHBP and GH-R *in vivo*. In transgenic mice expressing the bGH antagonist G119K-bGH, the concentration of IGF-I, the principal mediator of GH activity, could be correlated to

the percentage of ^{125}I -GH bound to serum. Since the labeled hormone can only bind to free GHBP, we believe that the concentration of free GHBP could be applied as a simple method to evaluate the potential utility of the use of GH antagonists in clinical therapy. G119K-bGH was suggested to be internalized in transgenic dwarf mice as 1:1 (GH:[GH-R]) complexes. Further work will be necessary to elucidate the mechanism(s) responsible for the ability of the G119K-bGH analog devoid of somatogenic activity to up-regulate the levels of the GH-R and GHBP (Chen *et al.* 1991a,b).

Acknowledgements

We thank Dr Albert F Parlow for the reagents for mouse GH RIA, the Pituitary and Hormone Distribution Program for the remaining hormones and reagents, Mrs R Cerven for maintaining the breeding colony of mice, and Ms M Hilt for expert secretarial assistance.

This work was supported by grants from Conicet and UBA, Argentina (D T) and from NIH HD20033 (A B) and the State of Ohio Eminent Scholar Program which includes a gift from Milton and Lawrence Goll, and Sensus Corp. (JJ K). D T is a career investigator from Conicet and is grateful to Fundación Antorchas for its support.

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Received 13 June 1997

Revised manuscript received 23 December 1997

Accepted 16 February 1998