Serum and liver cytosolic growth-hormone-binding proteins are antigenically identical with liver membrane ‘receptor’ types 1 and 2

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Studies with a panel of monoclonal antibodies (MAbs) reactive towards the presumptive rabbit liver growth-hormone (GH) receptor show that the rabbit serum GH-binding proteins share seven antigenic determinants (three at the hormone-binding site and four located elsewhere) with the liver cytosolic GH-binding proteins and the putative GH ‘receptors’ associated with the hepatocyte membrane. The rabbit serum binding proteins have an affinity for GH similar to the membrane GH receptors [for human GH, $K_a = 2.45 (\pm 0.15) \times 10^9 \text{ M}^{-1}$ (mean $\pm$ S.E.M., $n = 8$)] and high capacity relative to membrane ‘GH receptors’. Analogues of the postulated membrane ‘receptor’ subtypes 1 and 2 exist in the serum, but not subtype 3, which is also absent from liver cytosol. The serum and cytosolic binding proteins have identical cation-dependence properties; hGH binding is Ca^{2+} -dependent, whereas oGH binding is Ca^{2+} -independent. Affinity labelling of hGH-affinity-purified serum binding proteins with ^{125}I -hGH demonstrated a major GH-binding subunit, of M_r 55000, identical with the major component purified from membranes. In view of their high affinity and capacity, the serum binding proteins could control availability of GH to membrane receptors. It is suggested that the cytosolic binding proteins may be newly synthesized serum binding proteins. The existence of a close relationship between subsets of membrane-associated GH-binding sites, the serum GH-binding proteins and cytosolic GH-binding proteins dictates a reappraisal of earlier ligand-binding studies, which did not distinguish between binding-site subsets in the liver.

INTRODUCTION

We have previously reported the use of a panel of MAbs to the presumptive GH receptor in rabbit and rat liver [1,2] to demonstrate a close antigenic and possible ontogenic relationship between membrane ‘receptor’ subtypes 1 and 2 and the cytosolic GH-binding proteins that are present in large amounts in the rabbit liver [2,3]. We also reported antigenic mapping studies with these MAbs, on the basis of which we postulated the existence of three subtypes of rabbit liver GH receptor [2], one of which (type 3) was not recovered after Triton X-100 solubilization of liver microsomes (microsomal fractions), although it shared at least one antigenic determinant with the other subtypes [2].

Ymer and co-workers [4] recently reported the existence of a serum GH-binding protein in the rabbit and in man [5]. In the present paper we compare salient physico-chemical and antigenic features (with respect to our MAb panel) of these serum binding proteins with the cytosolic GH-binding proteins and with presumptive receptor subtypes 1, 2 and 3 in the rabbit liver.

MATERIALS AND METHODS

Materials

oGH (S-13 and I-3), hGH (I-1), oPRL-16, rabbit GH (AFP-684C) and rat PRL (B-3) were gifts from the National Hormone and Pituitary Program (Baltimore, MD, U.S.A.). hGH for receptor purification was

generously provided by the Human Pituitary Advisory Committee of Australia (Woden, A. C. T., Australia). Porcine insulin was from Connaught Laboratories (Toronto, Canada).

Hormones were iodinated by the lactoperoxidase method of Thorell & Johansson [6], as described previously [7], and fractionated on a Sephadex G-100 column. Rabbit anti-mouse immunoglobulins were purchased from DAKO immunoglobulins (Glostrup, Denmark).

Preparation of receptor and binding-protein-containing fractions

The liver receptor preparations used in the present study were derived from a three-stage purification of the GH receptor [7]. Studies on inhibition of [^{125}I]iodohormone binding reported previously [1,2] were carried out on crude microsomal (100000 g, 60 min) fractions of rabbit liver. The cytosolic preparations used in the present (and previous) studies [2,3] were the supernatants from the crude microsomal preparations, filtered through Whatman no. 541 filter paper to remove fat. Immunoprecipitation and inhibition experiments were also carried out with Triton X-100 (1%, v/v)-solubilized extracts of these microsomal membranes (300000 g/90 min supernatants) or with rabbit liver GH receptor partially purified from these extracts by differential affinity chromatography on an hGH affinity column [7]. GH-binding proteins were purified by the same affinity-chromatography procedure from serum,

Abbreviations used: GH, growth hormone (somatotropin); PRL, prolactin; the prefixes h and o refer to the human and sheep hormones respectively; MAb, monoclonal antibody; PEG, poly(ethylene glycol); DMSO, dimethyl sulphoxide; PMSF, phenylmethanesulphonyl fluoride; DTT, dithiothreitol; r.r.a., radioreceptor assay.

after addition of MgCl_2 to 10 mM. In this case, no Triton X-100 was added to the serum or purification buffers. Laemmli [8] gels of this material showed major bands at 52000 and 80000 M_r , and the degree of purification was 105-fold. These bands presumably contain additional protein impurities, since this preparation was less than 1% pure as assessed by specific binding activity.

Affinity cross-linking

This was performed with either hGH-affinity-purified serum binding protein or hGH-affinity-purified liver GH receptor. Binding protein at approx. 10 fmol/ml was incubated for 12–14 h with ^{125}I -hGH (300000 c.p.m./ml) in 20 mM-Hepes buffer [containing 0.1% Triton X-100, 10 mM- MgCl_2 , 0.2% bacitracin, leupeptin and pepstatin (10 $\mu\text{g}/\text{ml}$), 10 mM-benzamidine and 1 mM-PMSF] at 20 °C. Disuccinimidyl suberate (Pierce) was added as a 100-fold concentrate in DMSO, with vortex mixing, to give a final concentration of 0.5 mM. Binding protein was left 15 min at 4 °C to cross-link, then cross-linking was blocked by the addition of Tris to 20 mM, pH 7.4. The solution was centrifuged briefly to clarify, then MAb 263 was added to 1:5000 (v/v) final dilution in the Hepes cross-linking buffer. This was left 8–12 h at 4 °C, then goat anti-mouse second antibody was added to a final

dilution of 1:200, followed by a further 3 h incubation at 4 °C. Cross-linked, immunoprecipitated, ^{125}I -labelled complexes were harvested by centrifugation (2000 g, 20 min), then solubilized in Laemmli sample buffer with 10 mM-DTT and subjected to slab-gel electrophoresis by the method of Laemmli [8]. Gels were dried and autoradiographed with intensifying screens (Lightning-Plus; du Pont).

Serum

Serum was obtained from the arterial blood of mature male and female rabbits. After complete clotting at room temperature and 2 h at 4 °C to allow clot retraction, the blood was centrifuged at 4 °C. Serum and cytosol preparations were stored at -20 °C. The Ca^{2+} concentration in samples was determined by atomic-absorption spectrophotometry.

Assay methods

The cytosolic GH binding proteins and serum GH-binding proteins are PEG-soluble [2,4]. In order to perform receptor-binding assays by the standard methods [7], a precipitating MAb (MAb 1), which does not interfere with hormone binding [1], was added to the incubation mixtures at a 1:1000 dilution in r.r.a. buffer

Fig. 1. Titration of precipitating MAbs against (a) detergent-solubilized liver microsomes; (b) cytosolic GH-binding proteins, (c) female and (d) male serum GH-binding proteins

The ordinates show the antibody-precipitable specific binding as a percentage of PEG-precipitable specific binding. Points represent means of triplicate determinations with S.E.M. values indicated. At a final assay dilution of 1:5000 the concentrations of the MAbs were: $\circ$, MAb5 (8 $\mu\text{g}/\text{ml}$); $\blacklozenge$, MAb43 (4 $\mu\text{g}/\text{ml}$); $\bullet$, MAb1 (4 $\mu\text{g}/\text{ml}$); $\blacktriangle$, MAb2 (4 $\mu\text{g}/\text{ml}$); $\triangle$, MAb44 (4 $\mu\text{g}/\text{ml}$). For (a), (b), (c) and (d) respectively the final concentrations of GH-binding sites were 90, 63, 300 and 320 fmol/ml. Specific binding of ^{125}I -hGH was 11, 9, 24 and 30% respectively. Non-specific binding was 5, 18, 14 and 17% respectively.

Fig. 2. Inhibition of $^{125}\text{I-hGH}$ ($\blacktriangle$, $\circ$) or $^{125}\text{I-oGH}$ ($\bullet$) binding to (a) affinity-purified 'GH receptor', (b) liver cytosolic GH-binding proteins and (c) serum GH-binding proteins

Points represent means of triplicate determinations, with S.E.M. indicated. Inhibition by MAb263 ($\circ$, $\bullet$); inhibition by MAb7 ($\blacktriangle$). For (a), specific binding of $^{125}\text{I-hGH}$ and $^{125}\text{I-oGH}$ was 17 and 10% of added radioactivity

[7]. The binding-protein-hormone complexes were then PEG-precipitable. Our previous work [1] has shown that at least half of the affinity-purified rabbit 'GH receptor' is PEG-soluble, so to render the complexes completely PEG-precipitable, MAb 1 was also incorporated in inhibition assays with the affinity-purified 'receptor'.

Cytosol, serum and affinity purified 'GH receptor' preparations were pre-titrated for ^{125}I -hormone binding in a standard r.r.a. [7] (incorporating MAb 1), before precipitation, inhibition or Scatchard [9] experiments.

MAbs used in all experiments were purified from mouse ascitic fluid by $(\text{NH}_4)_2\text{SO}_4$ precipitation, followed by dialysis or gel filtration on Sephacryl S-300.

Inhibition of GH binding to cytosolic or serum binding proteins

A 100 μl portion of appropriately diluted serum, cytosol or affinity-purified rabbit GH receptor was pre-incubated overnight at 4 $^\circ\text{C}$ with serial dilutions of the test MAb (100 μl). Parallel serial dilutions of an unrelated MAb (anti-*Brucella*) were used as binding controls. A 100 μl portion of MAb 1 at 1:1000 was added to both test and control tubes. A 100 μl portion of $^{125}\text{I-hGH}$ or $^{125}\text{I-oGH}$ was then added (100000 c.p.m.), together with 100 μl of r.r.a. buffer or 100 μl of excess unlabelled oGH (1 μg) in the binding control tubes. Incubation was continued for 4 h at 22 $^\circ\text{C}$, followed by precipitation with PEG.

Precipitation of binding-protein-hormone or 'GH receptor'-hormone complexes by MABs

Titration of precipitating MABs against Triton X-100-solubilized receptor-hormone complexes was performed by the standard double-antibody method as detailed in reference [1]. Titration of precipitating MABs against cytosolic or serum binding proteins was performed by using PEG to separate bound and free binding proteins as follows. After affinity labelling the diluted preparations overnight at 4 $^\circ\text{C}$ with $^{125}\text{I-GH}$, serial dilutions of test and control MABs were added to the labelled complexes, with and without excess unlabelled hormone. The incubation was continued for 3 h at 4 $^\circ\text{C}$ and the reaction then stopped by PEG precipitation [1 ml of unlabelled bovine γ -globulins (0.2%), 1 ml of PEG 6000 (30%, w/v), before vortex-mixing and centrifugation at 1600 g for 25 min].

Determination of affinity constants

Binding affinities of serum and cytosolic GH-binding proteins were estimated by Scatchard [9] analysis of ^{125}I -hormone binding to pre-titrated serum or cytosol in the presence of increasing concentrations of unlabelled hormone. This was carried out as described previously [1,2,7], but with the incorporation of precipitating MAB 1 into the incubation mixture to enable PEG precipitation of binding-protein-hormone complexes from serum or

respectively. Non-specific binding was 23 and 21% for hGH and oGH respectively. hGH-affinity-purified receptor was added to a final concentration of 52 fmol/ml. For (b), specific binding of hGH and oGH was 9 and 8% of added radioactivity respectively. Non-specific binding was 20 and 16% respectively. Binding protein was at a final concentration of 50 or 200 fmol/ml. For (c), specific binding was 22 and 6% for hGH and oGH respectively. Non-specific binding was 17% and 20% respectively.

cytosol. The specific radioactivity of the radiolabelled hormone was calculated from the fractional incorporation of total added radioactivity into peak fractions eluted from the Sephadex G-100 column used in the iodination procedure. No correction was made for bindability of labelled hormone.

Production and characterization of the anti-(GH receptor) MAbs

This has been described in detail previously [1,2]. The MAbs do not cross-react with prolactin or insulin receptors in the rabbit or rat. MAbs 263, 7 and 44 inhibit components of GH binding to rabbit liver microsomes, cytosol and affinity-purified 'GH receptor' (see [1,2] and the Results section).

Independence of epitopes recognized by MAbs

MAbs were tested for mutual competition and for competition with GH for binding sites in rabbit liver microsomes. The inhibition assays were performed by the standard method [2,3] with either ^{125}I -Mab or ^{125}I -GH as ligand. In this way, epitopes recognized by MAbs, 1, 2, 5, 7, 263, 43 and 44 were shown to be different, although in some cases (detailed in [2]) the Mab-binding regions (as distinct from epitopes) overlap.

RESULTS

Comparative precipitation curves

Figs. 1(a)–1(d) show the titration curves for the panel of MAbs against solubilized liver microsomes (Fig. 1a), liver cytosol (Fig. 1b) and unpurified serum binding proteins (Figs. 1c and 1d). The curves for serum and cytosol are virtually identical. Corresponding curves are parallel, and the relative order of titres is the same for both preparations (Mab 43 $\geq$ Mab 5 > Mab 1 > Mab 2 > Mab 44). The curve for solubilized microsomes is not identical with the curves for cytosol or serum binding proteins, but the order of titres is preserved for the MAbs tested. The decrease in titre observed with detergent-solubilized 'GH receptor' (Fig. 1a) is attributable to the lower sensitivity of our double-antibody method and

Fig. 3. Scatchard [9] analysis of ^{125}I -hGH binding to male (●, ■) and female (▲) rabbit serum GH-binding proteins

The female serum and two different male sera were at 1:125 (v/v) dilution. Specific binding was 13 (●), 11 (■) and 14% (▲) of radioactivity added. Non-specific binding was 18, 17 and 15% respectively. The specific radioactivity of ^{125}I -hGH was 90 $\mu\text{Ci}/\mu\text{g}$.

Fig. 4. Competition for Mab 1-precipitable ^{125}I -hGH-binding sites in male rabbit serum

Serum was added to 1:125 (v/v) final dilution. (a) Specific binding of ^{125}I -hGH was 10%. The specific radioactivity of ^{125}I -hGH was 40 $\mu\text{Ci}/\mu\text{g}$. Displacement was by hGH I-1 (●), oGH-13 (▲), oPRL-16 (○) and porcine insulin (■). (b) Specific binding of ^{125}I -hGH was 9.4%. The specific radioactivity of ^{125}I -hGH was 64 $\mu\text{Ci}/\mu\text{g}$. Displacement was by hGH I-1 (●), rabbit GH AFP-684C (▲) and rat PRLB-3 (○). Points are means of triplicate determinations, with the S.E.M. indicated.

possibly to diminished reactivity of epitopes in the presence of detergent. A similar decrease in titre is observed when the double-antibody method is used with serum or cytosol, but in these cases, where detergent is not present, the slope of the titration curves is unchanged. A similar pattern is seen with affinity-purified 'GH receptor' derived from the solubilized microsomes [1]. Figs. 1(c) and 1(d) show the identity of antigenic profiles for male and female serum binding proteins.

Comparative inhibition curves

Mab 7 and Mab 263 inhibit components of GH binding to rabbit liver microsomes, affinity-purified preparations from these microsomes and the cytosolic binding proteins [1,2]. Figs. 2(a)–2(c) show inhibition of ^{125}I -hGH or ^{125}I -oGH binding to affinity-purified rabbit 'GH receptor' (Fig. 1a), cytosolic binding proteins (Fig. 1b) and serum binding proteins (Fig. 1c) by MAbs 263 and 7. Cytosolic and serum binding proteins were rendered PEG-precipitable by the inclusion of Mab 1 in the incubation mixture (see the Materials and methods section).

Fig. 5. (a, b) Ca^{2+} -dependence of hGH binding to serum and cytosolic binding proteins, and (c, d) Ca^{2+} -independence of oGH binding to serum and cytosolic binding proteins

MAB 1 was added to a final dilution of 1:5000 (v/v). (a) Serum at 1:100 (v/v) final dilution; (b) cytosol at 1:250 (v/v) final dilution. ●, Specific binding of ^{125}I -hGH with increasing concentrations of Ca^{2+} and without Na^{+} ; ▲, specific binding of ^{125}I -hGH with increasing concentrations of Ca^{2+} and with 150 mM- Na^{+} ; ○, inhibition of specific binding of hGH, by MAB 263 [1:5000 (v/v) final] at each Ca^{2+} concentration in the absence of Na^{+} ; ■, as for ● at zero added Ca^{2+} , but with the inclusion of 10 mM-EDTA. These two points (■, ●) do not differ significantly ($P > 0.10$, paired t test). The specific radioactivity of ^{125}I -hGH was approx. 64 μ Ci/ μ g. The specific binding was determined by displacement with excess unlabelled oGH (2 μ g/ml) for reasons given in the Results section. Points represent means of triplicate determinations, with the S.E.M. indicated. (c) Serum at 1:10 (v/v) final dilution; (d) Cytosol at 1:125 (v/v) final dilution. ●, Specific binding of ^{125}I -oGH with increasing concentrations of Ca^{2+} and without Na^{+} ; ○, inhibition of specific binding of oGH by MAB 263 [1:5000 (v/v) final] at each concentration of Ca^{2+} . The specific radioactivity of ^{125}I -oGH was approx. 77 μ Ci/ μ g. Specific binding was determined by displacement with excess unlabelled oGH (2 μ g/ml).

The same inhibition pattern is seen with both MABs for all three preparations. Just as for cytosol and affinity-purified rabbit 'GH receptor', MAB 263 does not inhibit ^{125}I -hGH binding, but inhibits up to 30% of ^{125}I -oGH binding to the serum binding proteins. So, with respect to the MAB 263 epitope, the serum binding proteins display the same differential binding of hGH and oGH as do the cytosolic binding proteins and the affinity-purified 'GH receptor' [3]. MAB 7 completely inhibits oGH or hGH binding to the serum binding proteins, so analogues of the postulated membrane receptor type 3 are not present in the serum, just as they are absent from cytosol (see the Discussion section).

The antigenic profiles are qualitatively the same in male and female sera, but there is a slightly greater amount of GH-binding protein in female serum (see below).

GH-binding capacity and affinity of the serum binding proteins

Scatchard analysis [9] was performed on serum from four mature male and four mature female rabbits (Fig. 3). The affinity of the serum binding protein for hGH was not significantly different ($P > 0.1$) in males and females [$2.45(\pm 0.15) \times 10^9$ M^{-1} (mean $\pm$ S.E.M., $n = 8$)]. The bin-

ding capacity was marginally higher in females [12980 ± 1320 fmol/ml of undiluted serum in females ($n = 4$); cf. 9380 ± 1070 fmol/ml in males ($n = 4$, $P < 0.05$)]. In comparison, the binding capacity of female liver microsomes is 8000 ± 1600 fmol/g of liver homogenized and the affinity for hGH is approx. 3×10^9 M^{-1} [7,10].

When serum was centrifuged at 100000 g for 1 h at 4 $^{\circ}C$, the binding capacity and affinity estimates were not decreased (paired t test; $P > 0.10$ at all points on displacement curves from which Scatchard plots were derived), so GH-binding sites on blood cells are not interfering in the assay systems.

Specificity of the serum-binding-protein binding sites

Fig. 4(a) shows the competition of oGH, hGH, oPRL and porcine insulin for ^{125}I -hGH-binding sites precipitable from male serum by MAB 1. As for membrane-associated GH-binding sites, PRL competes with the GHs, but with approx. 10-fold lower potency than oGH (see [7]). Insulin has no effect on GH binding to the serum binding proteins, even at concentrations as high as 10 μ g/ml. Fig. 4(b) shows the competition of rabbit GH and rat prolactin for ^{125}I -hGH-binding sites precipitable from male rabbit serum. The homologous ligand, rabbit GH,

Fig. 6. Electrophoresis of affinity-cross-linked binding proteins

S, hGH-affinity-purified binding proteins from serum, affinity-cross-linked to ^{125}I -hGH; M, hGH-affinity-purified binding proteins from rabbit liver membranes. + indicates parallel runs with $5\text{ }\mu\text{g}$ of unlabelled hGH/ml incorporated into the initial incubation mixture (—, no such incorporation). See the Materials and methods section for details of cross-linking and electrophoresis procedure.

competes with ^{125}I -hGH, but with 30-fold lower potency than unlabelled hGH (consistent with the findings of Hughes [11] and Hughes *et al.* [12] with rabbit liver membrane GH-binding sites).

Competition by rat prolactin is less than 20% when $10\text{ }\mu\text{g/ml}$ is added. Hughes [11] has shown that rabbit and rat PRL bind with similar low affinity to rabbit liver membranes. In view of the demonstrated similarity between serum- and membrane-associated GH-binding proteins, it is likely that rabbit and rat PRL will have comparably low affinities for the serum GH-binding proteins. If this be so, then the weak competition by rat PRL for GH-binding sites on the serum GH-binding proteins reflects the effective discrimination between homologous somatotrophic and lactogenic ligands by the rabbit serum binding proteins.

Ca^{2+} -dependence of GH binding to serum, cytosol and microsomes

hGH binds to serum (Fig. 5a) and cytosolic binding proteins (Fig. 5b) with virtually complete Ca^{2+} -dependence. Physiological concentrations of Na^+ can largely obviate the requirement for Ca^{2+} , so that, in the presence of Na^+ , a smaller Ca^{2+} -potentiated component is observed (Figs. 5a and b). By contrast, oGH binding to cytosolic or serum binding proteins is not Ca^{2+} -dependent or Ca^{2+} -potentiated (Figs. 5c and d). This is significant, since the Ca^{2+} -dependent hGH binding is completely displaceable by oGH. Apparently these ligands bind to the same sites, but with different cation-dependencies. hGH binding to liver microsomes is also Ca^{2+} -dependent, and oGH binding is not, in a manner parallel with that for serum and cytosolic binding proteins (results not shown).

Affinity labelling

Fig. 6 shows the close similarity between serum and liver-membrane-derived GH-binding proteins, in that both run at $55000\text{ }M_r$ (when 22000 is subtracted for the contribution of hGH), and, in both cases, the radioligand is displaceable by $5\text{ }\mu\text{g}$ of hGH/ml included in the original incubation mixture. Although total displacement was not obtained for the membrane-derived binding protein, residual binding in the presence of excess unlabelled hormone is small relative to the uncompeted-for $77000\text{ }M_r$ complex. The displaceable component at $45000\text{ }M_r$ probably represents hGH dimer. This implies that the hGH dimer is capable of binding to membrane and serum binding proteins, since the cross-linked hGH-receptor complex is immunoprecipitated with anti-receptor antibody before electrophoresis.

DISCUSSION

The ligand-binding studies hitherto performed by numerous workers (for review, see [13]) have, with rare exception, not addressed the question of the relevance of the GH-binding sites detected to the mediation of GH actions. Grichting and co-workers [14] did not detect gross differences in binding capacity of adipocytes sensitive or refractory to the insulin-like actions of GH, but could not rule out modulation of small subsets of receptors. Baxter & Zaltsman [15] showed that Somatomedin-C levels positively correlated with 'GH receptor' number in hypophysectomized rats, but negatively correlated in intact animals. The $20000\text{-}M_r$ variant of hGH is equipotent with $22000\text{-}M_r$ hGH in stimulating growth in hypophysectomized rats [16], but is a poor inhibitor of $22000\text{-}M_r$ hGH binding to rabbit liver microsomes [17]. Hughes *et al.* [12] showed that $20000\text{-}M_r$ hGH was bound with high affinity only by a subset of 'receptors' in the rabbit. To understand these results, the equation of subsets of GH-binding sites with metabolic actions is essential.

The existence of a close structural relationship between subsets of membrane-associated GH-binding sites (the putative 'receptor' subtypes 1 and 2 [2]), the serum GH-binding proteins and the cytosolic GH-binding proteins dictates a reappraisal of earlier ligand-binding studies, which could not discriminate distinct subsets of binding sites.

Our previous studies revealed antigenic heterogeneity

of the liver GH-binding sites [2], as well as selective ligand binding to the 'receptor' subtypes so defined [2,3]. Analogues of subtypes 1 and 2 exist in large amounts in liver cytosol [2,3]. The present work shows the existence in the circulation of corresponding proteins sharing epitopes with membrane 'GH receptor' subtypes 1 and 2 and cytosolic binding proteins. The common epitopes are located both in the hormone-binding region and elsewhere.

The existence of a 'type 3' GH receptor in rabbit liver membranes was inferred from our earlier antigenic mapping studies [2]. Inhibition of specific ^{125}I -oGH binding to rabbit liver membranes was not total, even at high MAb 7 concentrations (although maximum inhibition obtained could be augmented with MAb 263) [2]. MAb 7 could, however, totally inhibit rat ^{125}I -GH binding to rabbit liver membranes [2]. By contrast, MAb 7 totally inhibits ^{125}I -oGH binding to serum or cytosol. These results can be interpreted as evidence against the presence of the 'type 3' GH receptor in serum and cytosol, although it is present in liver microsomes before solubilization and in plasma membrane or endoplasmic-reticulum fractions derived from the microsomes [2,3].

The possibility emerges that the cytosolic GH-binding proteins may be newly synthesized serum binding proteins in transit to the cell membrane and thence the circulation, or even endocytosed serum binding proteins.

We have previously shown that the same antigens are associated with GH-binding sites in both endoplasmic-reticulum and plasma-membrane fractions from hepatocytes. It is now apparent that antigenically identical analogues of presumptive receptor subtypes 1 and 2 exist in the endoplasmic reticulum, cytosol, hepatocyte membrane and the circulation. These findings strongly implicate the liver as a site of synthesis of the serum GH-binding proteins.

In their studies of hGH heterogeneity, Stolar *et al.* [18] and other groups [19–21] were unable to demonstrate binding of exogenous GH to human plasma proteins, by gel filtration of plasma/GH co-incubation mixtures. However, these studies did not rule out the possibility of non-covalent interaction between hGH and plasma proteins. Our work supports the finding of Ymer *et al.* [4] that GH is bound with high affinity by a specific binding protein in the rabbit and in man [5]. Furthermore, the application of MAbs has enabled quantification and antigenic characterization of the serum GH-binding protein in the rabbit. In addition, we have shown that the major GH-binding subunit of both liver and serum that is immunoprecipitated by MAb 263 has an M_r of 55000.

The rabbit serum binding protein has high affinity for hGH ($2.45 \times 10^9 \text{ M}^{-1}$) and is present in substantial amounts in the serum ($\sim 13 \text{ pmol/ml}$ of undiluted female serum; cf. $\sim 8 \text{ pmol}$ of microsomal 'receptor'/g of female liver homogenized). Given the quantity of serum binding protein and the similar affinities of membrane and serum-binding-protein binding sites for GH, the conditions exist for the controlled exchange of GH from serum binding proteins to membrane receptors.

The binding capacity we measure in male or non-pregnant female rabbits is higher than that reported by Ymer *et al.* [4] [our value 13 pmol/ml (female); cf. 3.8 pfmol/ml [4]]. Ymer *et al.* [4] used a gel-filtration system at room temperature to separate bound from free ligand. This process may well involve some dissociation

of bound hormone, as evidenced by the small competition of oPRL for serum binding sites reported by these workers (although Ymer *et al.* [22] did report substantial displacement of ^{125}I -hGH from the cytosolic binding protein by oPRL when the gel-filtration method was used). We find substantial displacement of ^{125}I -hGH from the serum binding proteins by oPRL, albeit with 20-fold lower potency than hGH, as for membrane-associated 'GH receptors' [7]. This result is consistent with the similarity of the serum binding protein to membrane-associated 'GH receptors' and cytosolic binding proteins in four other respects (i.e. antigenic identity of binding and other sites, similar affinity, differential ligand binding [3] and identical cation-dependence).

Our estimate of the affinity of the serum binding protein for hGH [$2.45 (\pm 0.15) \times 10^9 \text{ M}^{-1}$] is close to values reported for membrane-associated 'GH receptor' [7,10] and cytosolic binding proteins [2,22].

It is significant that the serum binding proteins show the same differential binding of hGH and oGH as do the cytosolic and membrane-associated binding sites [3]. Our finding, that oGH and hGH binding to the same sites is Ca^{2+} -independent in the former case and Ca^{2+} -dependent in the latter case, supports our earlier observations of differential ligand binding [3]. In studies of GH-binding sites in the mouse liver, Ciccia-Torres & Dellacha [23] raised the possibility of shared binding sites that bound lactogenic and somatogenic ligands with different cation-dependencies.

Differential binding of GH variants to the serum binding proteins could control their particular availability to membrane GH receptors in target organs or the rate of clearance of different GHs from the circulation (Baumann *et al.* [24] have recently shown that the 20000- and 22000- M_r GHs have different metabolic half-lives). Discrimination of PRL and GH by the serum binding protein may be significant in this regard. Shiu's study with the MCF-7 and T-47D mammary-tumour-cell lines [25] showed that both hGH and hPRL bind with similar affinity to the PRL receptor in these human cells. Kleinberg & Todd [26] have found that hGH is a potent lactogen in subhuman primates. Sequestration of hGH by the circulating serum binding proteins could be necessary to prevent a permanent state of galactorrhoea. Conversely, a deficiency of serum GH-binding proteins could have a role in the aetiology of galactorrhoea.

The serum GH-binding proteins are likely to play a crucial role in regulating availability of GH to membrane-associated receptors or the cell interior.

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