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Soluble forms of the rabbit adipose tissue and liver growth hormone receptors are antigenically identical, but the integral membrane forms differ

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Cytosolic, detergent-solubilized and membrane-bound growth hormone (GH) receptors from rabbit adipose tissue and liver were tested for reactivity with a panel of monoclonal antibodies (MAbs). The cytosolic and detergent-solubilized forms of adipose tissue and liver GH receptors were identically reactive with four precipitating and two hormone-binding-site-directed MAbs. However, the membrane-bound form of the adipose receptor was 1000-fold less reactive with one binding-site-directed MAb (MAb 7) than the membrane-bound liver GH receptor. Reactivity with another inhibitory MAb (MAb 263) was identical for adipose tissue and liver membrane GH receptors. The relative potency of 22000-M_r and 20000-M_r forms of human GH was identical in assays with liver and adipose tissue membrane receptors. Thus, contrary to earlier suggestions, the discrepancy between the growth-promoting and insulin-like activities of 20000-M_r human GH cannot be rationalized by a difference in the affinity of this hormone for 'somatogenic' and 'metabolic' receptors when the comparison is made in the same species. Cross-linking studies showed that the major GH-binding subunit of liver and adipose tissue GH receptors had the same M_r (54000 ± 5000, reduced). The ligand-binding subunits of liver and adipose tissue receptors are identical by several criteria, but one epitope on the adipose tissue receptor appears to be masked upon membrane insertion, possibly by close association with a tissue-specific component. Tissue specificity may be determined by association of a ubiquitous GH-binding subunit with tissue-specific membrane components, rather than by differences in amino acid sequence.

INTRODUCTION

We have previously used a panel of MAbs reactive with the rabbit hepatic growth hormone (GH) receptor to demonstrate antigenic heterogeneity of the GH receptor in the rabbit liver [1] and to demonstrate the antigenic identity of a subset of liver GH receptors with the serum GH-binding protein [2]. We have since confirmed the identity of the extracellular domain of the cloned hepatic GH receptor and the serum GH-binding protein [3], and a single gene coding for a rabbit hepatic GH receptor has been cloned and expressed in COS cells [3]. Receptor types 1 and 2 [1] were present in the membranes of COS cells transfected with this gene, but type 3 was absent [3] (see the Discussion section). Recent work by Smith *et al.* [4] has revealed the existence of two related GH receptor mRNAs in mouse liver and adipose tissue, and these workers proposed alternative mRNA splicing as a mechanism for generating heterogeneous forms of the GH receptor and the serum GH-binding protein from a single gene. Earlier workers had suggested the existence of receptor subsets in adipocytes [5]. To determine whether antigenic heterogeneity exists among GH receptors in non-hepatic tissues and to discover whether the adipose and liver receptors are antigenically related, we have tested membrane-bound, cytosolic and detergent-solubilized forms of the GH receptors from both tissues for reactivity with the panel of monoclonal antibodies (MAbs). To detect possible macromolecular correlates of antigenic differences detected in the membrane-bound forms of the GH receptor, cross-linking studies were carried out on membrane GH receptors from both tissues.

Smal *et al.* [6] suggested that the attenuated insulin-like activity of the 20000-M_r variant form of hGH could be explained by the lower affinity of this hormone for the adipose tissue receptor relative to its affinity for a somatogenic receptor, but the comparisons carried out to date have not been made with adipose tissue and somatogenic receptors from the same species [6]. We therefore investigated the relative potencies of 22000-M_r hGH and 20000-M_r hGH for liver membrane and adipose tissue membrane GH receptors in both the rabbit and the rat. The results bear on the question of whether GH receptors in different tissues are products of the gene which has been cloned from the rabbit liver [3] and on the structural basis for GH receptor heterogeneity observed within the liver and between tissues.

MATERIALS AND METHODS

Materials

Recombinant-DNA-derived hGH was a gift from Genentech Inc. (San Francisco, CA, U.S.A.). The pituitary-derived 20000-M_r variant of hGH was generously donated by Dr. G. E. Chapman and was purified in his laboratory (Department of Biochemistry, University of Auckland, Auckland, New Zealand). 20000-M_r hGH from this source has been shown independently to electrophorese identically with recombinant-DNA-derived 20000-M_r hGH [7]. Sheep GH (oGH I-1 or oGH-14) was kindly provided by the National Hormone and Pituitary Program (Baltimore, MD, U.S.A.). hGH and oGH were iodinated by the lactoperoxidase method of Thorell & Johansson [8] as described previously [1]. Iodinated hormones were fractionated on a Sephadex G-100 column. Specific radioactivities were in the range 50–150 μCi/μg.

Abbreviations used: GH, growth hormone (somatotropin); PRL, prolactin; the prefixes h and o refer to the human and sheep hormones respectively; MAb, monoclonal antibody; PMSF, phenylmethanesulphonyl fluoride; EGS, ethylene glycolbis(succinimidyl succinate).

Preparation of liver and adipose tissue microsomes and cytosol

Liver microsomal membranes were prepared by the method described previously [9] from fresh tissue from non-pregnant female or male rabbits, except that additional proteinase inhibitors [10 mM-benzamidine, 3 mM-aminoacetonitrile hydrochloride and 1 mM-phenylmethanesulphonyl fluoride (PMSF) (Sigma, St. Louis, MO. U.S.A.)] were added to the homogenization buffer. Microsomes were resuspended in 1 ml of the bivalent-cation-free storage buffer of Haro *et al.* [10] for each gram of tissue homogenized. The adipose tissue microsomes were prepared by the same method from fresh tissue, but were resuspended in 0.2 ml of storage buffer for each gram of tissue homogenized. Microsomes were stored at −20 °C. The cytosol preparations used in these studies were the 100000 *g* supernatants of liver or adipose tissue microsomal pellets. Membrane-bound receptors were solubilized by resuspension and stirring overnight at 4 °C in 25 mM-Tris (pH 7.6)/1% Triton X-100 (Boehringer, Mannheim, Germany), containing 1 mM-PMSF. Soluble extract was obtained by centrifugation for 90 min at 100000 *g*.

Assay methods

The methods for the immunoprecipitation and inhibition assays used in this study have been described previously [1,11]. In this study, the double antibody method (as used in [11]) was employed for the precipitation assays. Briefly, the soluble receptor preparations were labelled by overnight incubation with ^{125}I-labelled hGH. Serial dilutions of the MAbs were then added and incubated for 3 h at 4 °C, followed by addition of a rabbit anti-mouse immunoglobulin (DAKO, Glostrup, Denmark) and diluted mouse serum. Incubation was continued for 90 min at 22 °C before the complexes were pelleted by centrifugation at 1600 *g*. A parallel set of tubes also contained unlabelled oGH-14 at 1 μg/ml to enable determination of specific binding to somatogenic receptors at each dilution of precipitating MAb.

In assays designed to measure the relative potency of 22000-M_r hGH and 20000-M_r hGH for somatogenic receptors, rather than for a mixed population of somatogenic and lactogenic receptors, prolactin (PRL) receptors were blocked by addition of excess oPRL-16 (0.5 μg/ml) to each assay tube. This concentration of PRL binds minimally to GH receptors, but completely occupies PRL receptors (see legend to Fig. 5).

Determination of affinity constants

Affinity of detergent-solubilized and microsomal GH receptors for hGH was estimated by Scatchard analysis [12] of ^{125}I-labelled hGH binding as described previously [13]. To ensure that the affinity of somatogenic rather than lactogenic sites was measured, oPRL-16 was added to all assay tubes at 0.5 μg/ml (final concentration of 100 ng/ml) to block PRL receptors. Data analysis and curve fitting were performed using the EBDA and LIGAND programs [14] from Elsevier Biosoft (Cambridge, U.K.).

Measurement of GH binding to IM-9 cells

Human IM-9 cells were grown at 37 °C in a humidified atmosphere of CO_2/air (1:19) in RPMI 1640 medium (Flow Laboratories, McLean, VA, U.S.A.) supplemented with 10% (v/v) foetal calf serum (Commonwealth Serum Laboratories, Brisbane, Australia). For assay of GH binding, cells from late log phase cultures were pelleted by centrifugation and then resuspended to 10^7 cells/ml in a buffer containing 25 mM-Tris, 150 mM-NaCl, 2 mM-Mg^{2+} and 0.1% BSA (Sigma), pH 7.6. To each 0.3 ml portion of cell suspension was added 0.1 ml of ^{125}I-hGH (100000 c.p.m.) and 0.1 ml of buffer containing unlabelled hGH at a range of concentrations from 0 to 10 μg/ml. Incubation was for 3 h at 25 °C, with shaking. The assay was terminated by adding 2 ml of ice-cold buffer followed by centrifugation at 1600 *g*.

Monoclonal antibodies

The production and characterization of MAbs to the GH receptor have been described previously [1,11,15]. All MAbs react with epitopes on the extracellular domain of the GH receptor.

The nature of epitopes recognized by the MAbs

The effect of inhibitory MAbs 7 and 263 on GH binding has been described elsewhere [1,2,11,15]. The epitopes recognized by these MAbs appear to be peptide determinants, since extensive treatment of the soluble affinity-purified GH receptor with a variety of glycosidases did not destroy the epitopes recognized by either MAb. Likewise, treatment of liver microsomal GH receptors with periodate and borohydride according to the method of Woodward *et al.* [16] did not destroy epitopes recognized by MAbs 263 or 7, although the affinity of MAb 7 for its epitope was decreased by 10-fold, suggesting a glycoprotein epitope. Furthermore, phosphate groups do not appear to form part of the epitopes, since treatment with alkaline phosphatase at pH 8.8 did not affect MAb reactivity (R. Barnard & M. J. Waters, unpublished work). Two of the four precipitating MAbs used in the present work (MAbs 1 and 2), have also been shown to recognize protein rather than carbohydrate determinants, since their reactivity was unaffected by treatment of the receptor with glycosidases.

Affinity cross-linking

^{125}I-hGH was cross-linked to microsomal membranes prepared from rabbit adipose tissue and liver, using ethylene glycolbis-(succinimidyl succinate) (EGS; Pierce) as follows. A 1 ml portion of microsomes was suspended in 30 ml of ice-cold Hepes buffer (pH 7.5) with 10 mM-Mg^{2+}. Microsomes were pelleted by centrifugation and then resuspended in 1 ml of Hepes buffer. Duplicate tubes with and without excess unlabelled oGH were used for each tissue. To 100 ml of optimally diluted microsomes was added 100 μl of ^{125}I-hGH (approx. 150000 c.p.m.), together with 300 μl of Hepes buffer or 200 μl plus excess unlabelled oGH-14 at a final concentration of 2 μg/ml. The mixture was incubated overnight at 4 °C with shaking. EGS was added as a 20-fold concentrate in dimethyl sulphoxide to give a final concentration of 0.5 mM. The cross-linking reaction was allowed to proceed for 15 min at 4 °C and was then stopped by the addition of Tris (pH 7.6) to a final concentration of 20 mM. Samples were then pelleted in a Microfuge, dissolved in Laemmli sample buffer with 10 mM-dithiothreitol and subjected to slab-gel electrophoresis by the method of Laemmli [17]. Gels were dried and autoradiographed with intensifying screens (Lightning Plus, Du Pont).

RESULTS

Reactivity of precipitating MAbs with GH-binding proteins from rabbit liver and adipose tissue cytosol

Four Mabs (Mabs 1, 2, 5 and 43) were tested for their ability to precipitate GH-binding proteins from adipose tissue and liver cytosol (Figs. 1*a* and 1*b*). The order of titres was the same for both adipose tissue and liver cytosolic binding proteins (MAb 43 > MAb 1 > MAb 5 > MAb2), and the titre for each MAb was the same against adipose and liver cytosolic binding proteins.

Reactivity of precipitating MAbs with detergent-solubilized GH-binding proteins from adipose tissue and liver membranes

Four precipitating MAbs were tested for their ability to precipitate GH-binding proteins, solubilized using Triton X-100,

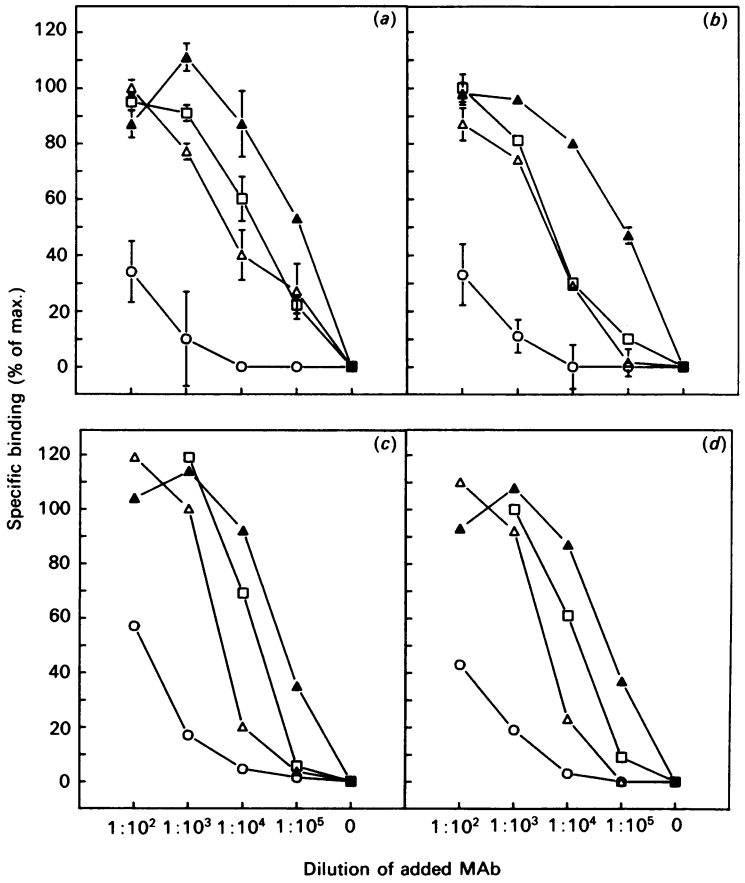

Fig. 1. Titration of precipitating MAbs against (*a*) adipose tissue cytosol, (*b*) liver cytosol, (*c*) detergent-solubilized adipose tissue microsomes and (*d*) detergent-solubilized liver microsomes

The ordinates show the antibody-precipitable specific binding as a percentage of the maximum precipitable specific binding. Points represent means of triplicate determinations with S.E.M. values indicated. The MAbs used were MAb 43 (▲), MAb 1 (□), MAb 5 (△), and MAb 2 (○). The final concentration of GH-binding sites in both liver and adipose cytosol was 60 fmol/ml. Specific binding (as determined by addition of oGH-14 at 10 μg/ml to a duplicate set of tubes) was 10% of added ^{125}I-hGH for both adipose tissue and liver cytosol; non-specific binding was 10% and 18% for liver and adipose tissue cytosols respectively. Specific binding was 7% and 6% for solubilized liver and adipose tissue microsomes respectively; non-specific binding was 7% for both solubilized liver and adipose tissue microsomes. The concentration of MAb protein was standardized to 5 mg/ml before addition to the assay at the indicated dilution.

from adipose tissue and liver microsomal membranes. As observed with the naturally soluble GH-binding proteins in adipose tissue and liver cytosol, the MAbs were identically reactive with detergent-solubilized binding proteins from either tissue. The order of titres was the same for both tissues, as were the absolute titres of each antibody tested (Figs. 1*c* and 1*d*).

Inhibition of oGH binding to integral membrane GH receptors from adipose tissue and liver by MAb 263

We previously reported that MAb 263 inhibits approx. 50% of oGH binding to rabbit liver membranes [1]. In the present work, MAb 263 was tested for inhibition of ^{125}I-oGH binding to microsomal membranes prepared from liver and adipose tissue from the same rabbit. MAb 263 was found to inhibit oGH binding to microsomes from both tissues in an identical fashion (Fig. 2*a*). The same result was obtained whether the tissue was obtained from male or female rabbits. Clearly, adipose tissue membrane GH-binding sites are identical to liver membrane binding sites with respect to epitopes recognized by MAb 263, in that only half of the sites can be blocked by MAb 263.

Difference in inhibition of GH binding to liver and adipose tissue membranes, and absence of this difference in the soluble form of the receptor

Inhibition of oGH-displaceable ^{125}I-hGH binding to adipose tissue and liver membranes was examined. The inhibitory titre of MAb 7 was at least 1000-fold lower against adipose tissue membranes relative to liver membranes (Fig. 2*b*). Significantly, this difference was not observed when the membrane-bound receptors were solubilized with Triton X-100 (Fig. 3*a*) or when the same experiment was conducted with soluble cytosolic GH-binding protein from each tissue (Fig. 3*b*). The inhibition curves were not significantly different and inhibition was total, as reported previously for soluble forms of the liver GH receptor [1,2,11,15].

Investigation of the yield of GH-binding sites solubilized from adipose tissue membranes

To demonstrate that the majority of GH-binding sites had been removed from the adipose tissue membrane by Triton X-100,

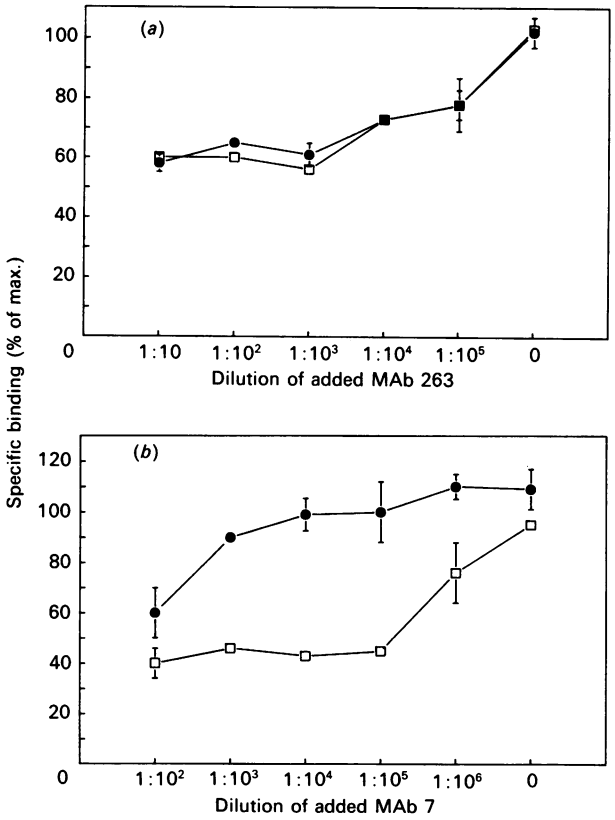

Fig. 2. Inhibition by (*a*) MAb 263 and (*b*) MAb 7 of (*a*) ^{125}I-oGH binding and (*b*) ^{125}I-hGH binding to adipose tissue (●) and liver (□) membranes

(*a*) Points represent means of triplicate determinations, with S.E.M. values indicated. The added concentration of GH-binding sites was 60 fmol/ml for adipose tissue membranes and 96 fmol/ml for liver membranes. Specific binding was 4% and 20% of added radioactivity for adipose tissue and liver respectively (as determined by addition of oGH-14 at 5 μg/ml to a parallel set of tubes at each antibody dilution). Non-specific binding was 8 and 10% for adipose tissue and liver respectively. (*b*) Points represent means of triplicate determinations, with S.E.M. values indicated. The added concentration of GH-binding sites was 30 fmol/ml for adipose tissue membranes and 15 fmol/ml for liver membranes. Specific binding (as determined by addition of 5 μg of oGH-14/ml to a parallel set of tubes at each antibody dilution) was 7 and 20% of added radioactivity for adipose tissue and liver membranes respectively. Non-specific binding was 16% and 19% of added ^{125}I-hGH for adipose tissue and liver respectively. The high non-specific binding with ^{125}I-hGH as ligand is due to binding to PRL receptors, which are abundant in female liver and adipose tissue membranes. The binding to PRL receptors is not displaceable by oGH, the ligand used in these experiments to detect binding to GH receptors only.

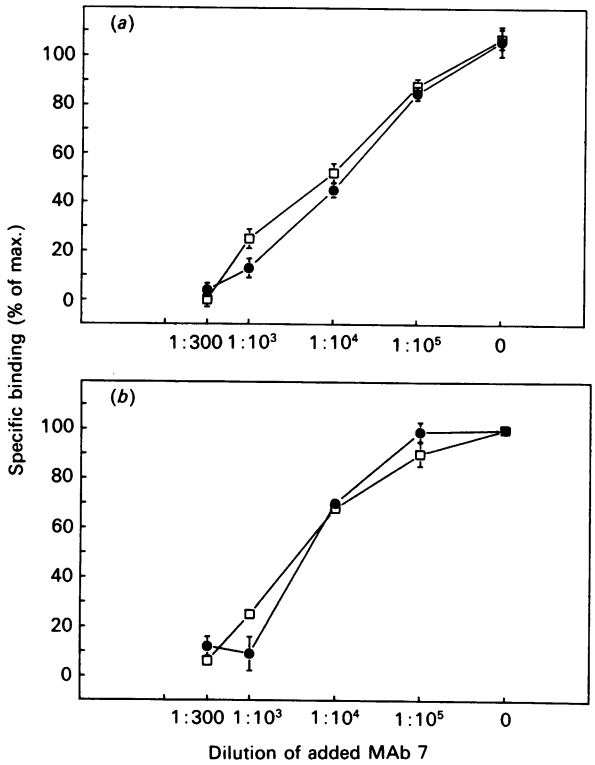

Fig. 3. Inhibition by MAb 7 of ^{125}I-hGH binding to (*a*) detergent-solubilized adipose tissue (●) and liver (□) membranes, and (*b*) cytosolic GH-binding proteins from adipose tissue (●) and liver (□)

Points represent means of triplicate determinations, with S.E.M. values indicated. The added concentration of GH-binding sites was 30 fmol/ml and 60 fmol/ml for adipose tissue and liver cytosol respectively. The added concentration of GH-binding sites was 30 fmol/ml for both detergent-solubilized adipose tissue and liver microsomes. Specific binding to solubilized adipose tissue and liver microsomes was 4% and 5% respectively of added ^{125}I-hGH. Non-specific binding to adipose tissue and liver cytosol was 5% and 9% respectively of added ^{125}I-hGH.

Scatchard analyses were carried out on adipose tissue membranes before and after detergent solubilization. Scatchard analyses revealed a single class (with respect to kinetic parameters) of high-affinity GH-binding sites in rabbit adipose tissue membranes (Fig. 4*a*). Fig. 4(*b*) shows that additional GH-binding sites become available after solubilization of the adipose tissue membrane, and the affinity of the solubilized sites was significantly greater than that of the membrane-bound GH receptor. A similar phenomenon has been observed after solubilization of PRL receptors, and has been attributed to the appearance of 'cryptic' receptors [18,19]. Since the yield of soluble receptors was at least 100%, it was considered that a representative sample of the membrane-bound adipose tissue GH-binding proteins was being tested in the detergent-solubilized form.

Relative potency of 20000-M_r and 22000-M_r hGH for adipose tissue and liver membrane GH-binding sites

It was considered possible that the antigenic difference between the hormone-binding regions of membrane forms of adipose tissue and liver receptors might be reflected by differences in hormone binding. To investigate this possibility, the relative potencies of 22000-M_r hGH and 20000-M_r hGH for somatogenic binding sites in liver and adipose tissue membranes from both rabbit and rat were determined. In these experiments, lactogenic (PRL) receptors were blocked by the inclusion of unlabelled oPRL at a final concentration of 0.1 μg/ml. The latter precaution is essential, since at least half of the hGH-binding sites in rabbit adipose tissue are PRL rather than GH receptors (Fig. 5). The relative potencies of 20000-M_r hGH and 22000-M_r hGH were the same for liver and adipose tissue somatogenic binding sites in microsomal membrane preparations from the rabbit (Fig. 6*a*) or the rat (Fig. 6*b*). In all cases, the 20000-M_r hGH was approx. 10 times less potent than 22000-M_r hGH in competing for somatogenic hGH binding sites. However, we have confirmed the finding of Smal *et al.* [6] that the affinity of 20000-M_r hGH is only slightly lower than the affinity of 22000-M_r hGH for the GH receptor on human IM-9 lymphocytes (Fig. 7).

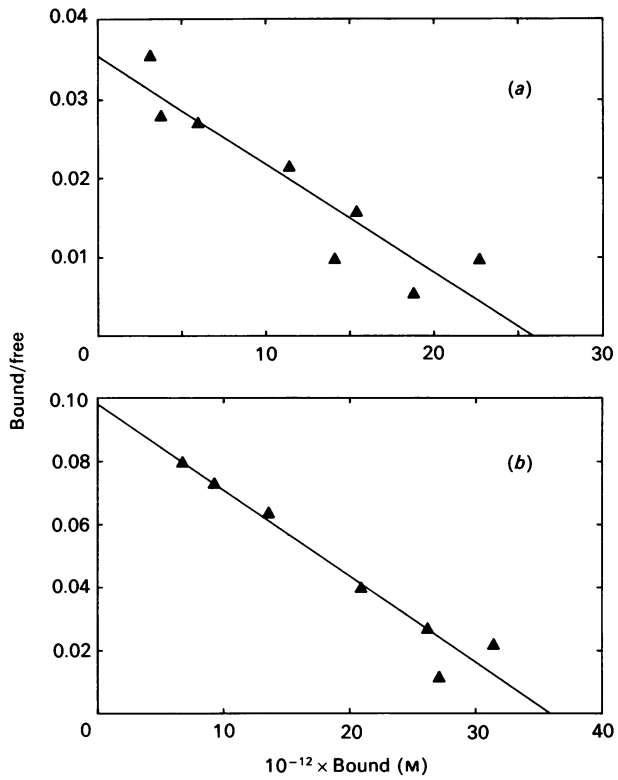

Fig. 4. Scatchard analysis of ^{125}I-hGH binding to adipose tissue membranes (*a*) and to detergent (Triton X-100)-solubilized adipose tissue membranes (*b*) from the same male rabbit

PRL receptors were blocked by the addition to all tubes of oPRL-16 at a concentration of 0.5 μg/ml. Membranes were added at a 1:2 (w/v) dilution. The material used in (*b*) was solubilized from the same mass of microsomes as used in (*a*). For membranes (*a*), $K_a = 1.5 \times 10^9$ M^{-1}; for solubilized membranes (*b*), $K_a = 2.9 \times 10^9$ M^{-1}. Specific binding to membranes and to solubilized membranes was 3.4% and 10% respectively, and non-specific binding was 6% and 5% respectively. The specific radioactivity of ^{125}I-hGH was 70 μCi/μg.

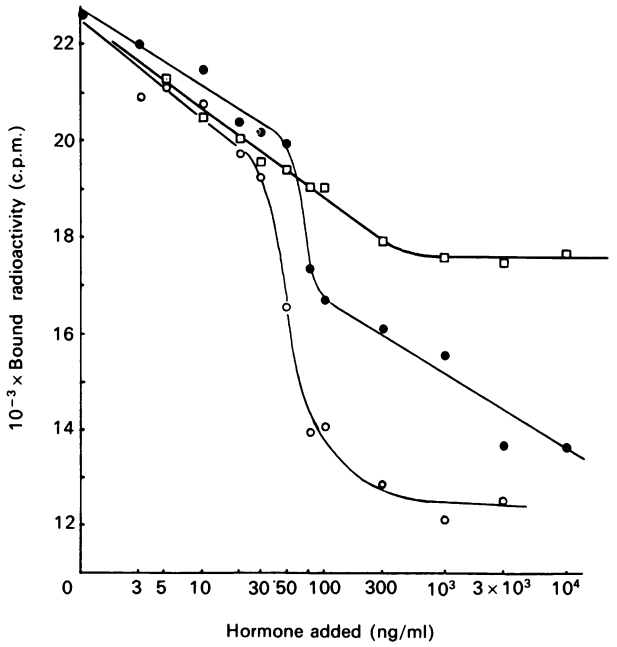

Fig. 5. Demonstration of PRL receptors in rabbit adipose tissue microsomes

The curves show displacement of ^{125}I-hGH binding to adipose tissue membranes by hGH (○), oGH (□) and oPRL (●). oGH binds only to GH receptors, hGH binds with high affinity to both GH and PRL receptors, whereas oPRL has high affinity for PRL receptors but also, at concentrations greater than 0.1 μg/ml, binds to GH receptors. Maximum specific binding was 12% of added ^{125}I-hGH.

Cross-linking studies

To investigate the possibility of size differences between the ligand-binding subunits of adipose tissue membrane and liver membrane GH receptors, ^{125}I-hGH was cross-linked to microsomal membranes prepared from rabbit adipose tissue and liver using the cross-linking agent EGS. As shown in Fig. 8, after reduced SDS/gel electrophoresis and autoradiography, major hGH-binding protein bands were observed at positions corresponding to M_r 130000 and 54000 for both liver and adipose tissue. However, in the adipose tissue, additional bands around M_r 130000 were also observed.

DISCUSSION

Although heterogeneity of somatogenic GH-binding sites in the liver was indicated by earlier studies [1,2,11,15,20–23], the structural basis for the observed heterogeneity remained to be elucidated. The need to address this question became more urgent after the cloning and expression of the cloned GH receptor by Leung *et al.* [3] and the demonstration [4] of two related mRNA species in the mouse liver and adipose tissue which hybridize with a GH receptor cDNA probe based on the sequence published by Leung *et al.* [3]. The short RNA transcript was shown to be translated into a protein presumed to be the GH-binding protein precursor, whereas the long transcript was translated into the full-length receptor [4]. It seemed reasonable to conclude that a single gene was encoding the serum GH-binding protein precursor and the full-length GH receptor in the liver as well as in the adipose tissue. Consistent with this view is our finding that the soluble forms of the GH receptor from adipose tissue and liver are antigenically identical with respect to several protein epitopes recognized by a panel of inhibitory and precipitating MAbs.

However, in contrast, the integral membrane form of the adipose tissue GH receptor differed markedly from the liver membrane forms of GH receptor in its reactivity with the inhibitory MAb 7. The inhibitory titre against adipose tissue membrane GH receptors was approx. 1000-fold lower than the inhibitory titre against the liver membrane GH receptor. The protein determinant was not destroyed or chemically modified upon membrane insertion, since it re-appeared upon removal of the receptor from the membrane by detergent solubilization, and was also present in the soluble form of the GH-binding protein found in adipose tissue cytosol. It is therefore suggested that the reactivity of this protein epitope may be modulated by the membrane environment of the receptor, possibly by occlusion of the MAb 7 epitope by an associated integral membrane component. Membrane-induced conformation changes or differences in receptor orientation are alternative possibilities that could also result from interaction between a tissue-specific membrane component and the GH-binding subunit of the receptor. The reactivity of another protein epitope close to the ligand-binding site (that recognized by MAb 263) was not modulated by the membrane environment and was equally reactive in adipose tissue and liver membrane GH receptors (see model, Fig. 9). The same heterogeneity with respect to the presence of the epitope recognized by MAb 263 was found for

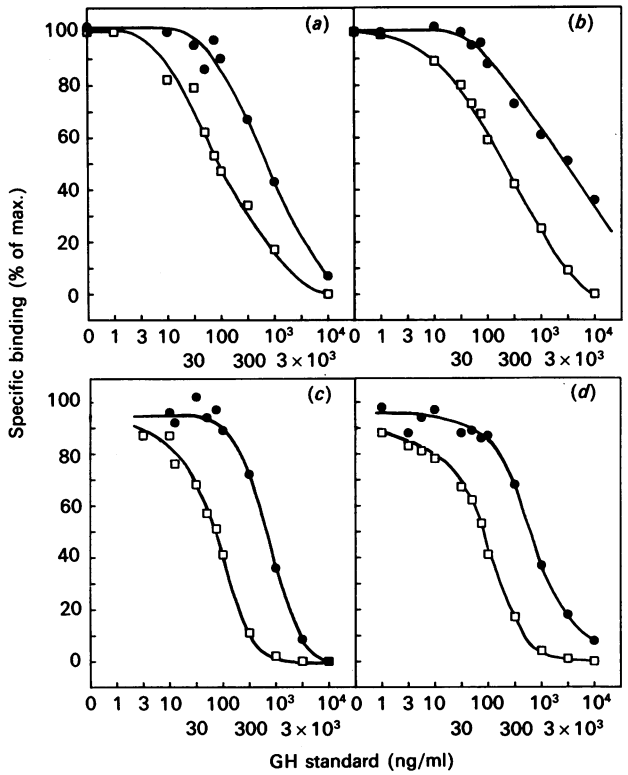

Fig. 6. Relative potency of 20000-M_r and 22000-M_r hGH in competing for ^{125}I-labelled 22000-M_r hGH-binding sites in adipose tissue and liver membranes from the rat (*a*, *b*) and the rabbit (*c*, *d*)

(*a*), (*c*) Displacement of ^{125}I-hGH binding to adipose tissue membranes. Displacement was by 22000-M_r-hGH (□) and 20000-M_r (●) for both tissues in both species. PRL receptors were blocked by the addition to all tubes of oPRL-16 at 0.5 μg/ml. Specific binding to rabbit liver and adipose tissue membranes was 10% and 6% respectively, and non-specific binding was 3% and 4% respectively (lower than in Fig. 2, since PRL receptors have been blocked in this experiment). Specific binding to rat liver and adipose tissue membranes was 18% and 6% respectively, and non-specific binding was 11% and 9% respectively.

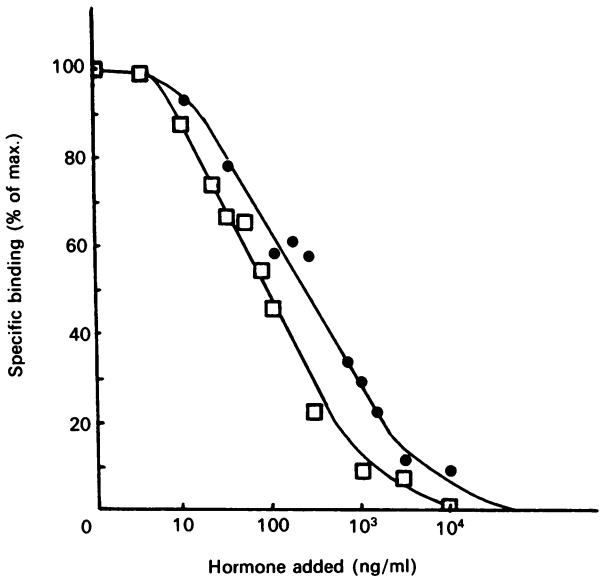

Fig. 7. Relative potency of 20000-M_r (●) and 22000-M_r (□) hGH in competing for ^{125}I-labelled 22000-M_r-hGH binding to the GH receptor of human IM-9 lymphocytes

Specific binding was 4% of added ^{125}I-HGH; non-specific binding was also 4%. IM-9 lymphocytes from a late log phase culture were suspended in 25 mM-Tris/150 mM-NaCl/2 mM-Mg^{2+}/0.1% BSA (pH 7.6) to a concentration of 10^7 cells/ml. The cells were incubated with ^{125}I-hGH and a range of concentrations of unlabelled hormones for 3 h at 25 °C with shaking.

both adipose tissue and liver membrane GH receptors. Hence at least the type 1 and type 2 GH-binding sites [1] are also present in the adipose tissue membranes. Since the epitope recognized by MAb 263 is a peptide determinant, this heterogeneity possibly results from specific proteolysis.

We reported previously that a significant proportion of GH binding to somatogenic sites in the rabbit liver was not inhibitable by MAb 7 [1,11,15], although binding to soluble receptors extracted from the membrane was totally inhibited. A similar phenomenon, that is occlusion of the MAb 7 reactive epitope by neighbouring integral membrane components, might account for the inability of MAb 7 to inhibit binding to a subset of liver receptors which were defined as type 3 GH receptors [1]. Of relevance to the possible basis for the antigenic heterogeneity within and between tissues is the antigenic character of the GH-binding sites expressed in the COS-7 monkey kidney cell line [3]. In the COS cells transfected with the single GH receptor gene from the rabbit, only types 1 and 2 GH-binding sites are expressed in the membrane, and GH binding can be completely inhibited by MAb 7. It is possible that integral membrane components which occlude the MAb 7 epitope on a proportion of the GH-binding proteins are absent from the COS cell membrane. Additional work will be necessary to test this

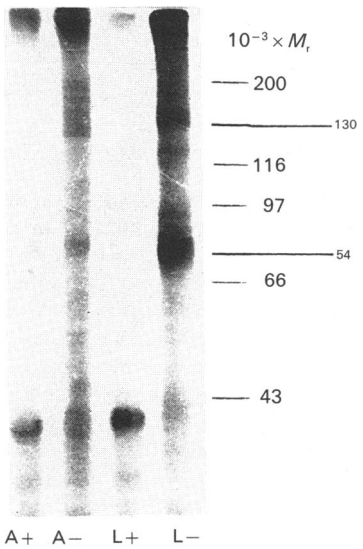

Fig. 8 Electrophoresis of affinity-cross-linked binding proteins solubilized from liver and adipose tissue membranes

A, Adipose tissue membrane GH-binding proteins cross-linked to ^{125}I-hGH; L, liver membrane GH-binding proteins cross-linked to ^{125}I-hGH. +indicates parallel runs with incorporation of 5 μg of unlabelled oGH-14/ml. See the Materials and methods section for details of the cross-linking procedure. The large numbers in the right hand column show the M_r values of the standards; the small numbers show the M_r values of the major GH-binding proteins (with the M_r of hGH subtracted).

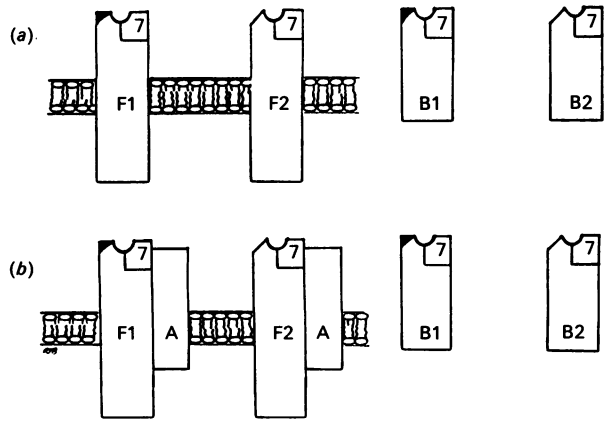

Fig. 9. Model for membrane-bound and soluble forms of GH-binding proteins in rabbit liver (*a*) and adipose tissue (*b*)

F, Full-length GH-binding subunit; B, released soluble binding protein; A, postulated adipose-specific integral membrane component of unknown structure, which either occludes the MAb 7 epitope or changes the receptor conformation or orientation (see the Discussion section). 7, epitope recognized by MAb 7; ◤, epitope recognized by MAb 263. Types 1 and 2 binding proteins (as defined by the presence or absence of the epitope recognized by MAb 263) are shown.

hypothesis, but other workers [23,24] have presented evidence consistent with complexes between a GH-binding subunit and a non-ligand-binding subunit.

In the present study, cross-linking studies were carried out on liver and adipose tissue membranes. The overall pattern of bands was similar for membranes from both tissues. The minor differences (e.g. the additional bands in the 100000-M_r region in adipose tissue) may result from differential glycosylation.

The comparative behaviour of the 22000- and 20000-M_r forms of human GH was of interest, since it has been suggested [6] that the discrepancy between the growth-promoting and insulin-like actions was explained by the low potency of 20000-M_r hGH for adipose tissue receptors relative to its potency for the IM-9 lymphocyte receptor. The latter was assumed to be a typical somatogenic receptor. However, we found that the relative potencies of 22000-M_r and 20000-M_r hGHs are the same for liver and adipose tissue membranes from either the rabbit or the rat. Thus the discrepancy between the high potency of 20000-M_r-hGH in stimulating somatomedin production [25] and its poor potency in assays for insulin-like activity [7] cannot be explained by a difference in affinity for 'somatogenic' and 'metabolic' receptors. The antigenic difference between the membrane forms of adipose tissue and liver receptors was not reflected by differential reactivity with 22000-M_r and 22000-M_r hGH.

In conclusion, comparison of GH receptors in different tissues has permitted us to postulate a structural basis for the antigenic heterogeneity of GH receptors. Consistent with the existence of a single gene coding for the GH-binding unit of the GH receptor, tissue specificity may be determined by association of a ubiquitous GH-binding subunit with tissue-specific membrane components, rather than by differences in primary amino acid composition. The nature of the membrane components remains to be elucidated, but cytoskeletal elements or structures involved in tissue-specific signal transduction are candidates worthy of further investigation.

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REFERENCES

1. Barnard, R., Bundesen, P. G., Rylatt, D. B. & Waters, M. J. (1985) Biochem. J. **231**, 459–468
2. Barnard, R. & Waters, M. J. (1986) Biochem. J. **237**, 885–892
3. Leung, D. W., Spencer, S. A., Cachianes, G., Hammonds, R. G., Collins, C., Henzel, W. J., Barnard, R., Waters, M. J. & Wood, W. I. (1987) Nature (London) **330**, 537–543
4. Smith, W. C., Linzer, D. I. H. & Talamantes, F. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 9576–9579
5. Goodman, H. M. & Kostyo, J. L. (1981) Endocrinology (Baltimore) **108**, 553–558
6. Smal, J., Closset, J., Hennen, G. & De Meyts, P. (1986) Biochem. Biophys. Res. Commun. **134**, 159–165
7. Tinsley, F. C., Grinnan, E. L., Baker, S. H., Powell, J. G., Bemis, K. G. & Shaar, C. J. (1986) Bichem. Biophys. Res. Commun. **138**, 342–348
8. Thorell, J. I. & Johansson, B. G. (1971) Biochim. Biophys. Acta **251**, 363–369
9. Tsushima, T. & Friesen, H. G. (1973) J. Clin. Endocrinol. Metab. **37**, 334–337
10. Haro, L. S., Collier, R. J. & Talamantes, F. J. (1984) Mol. Cell. Endocrinol. **38**, 109–116
11. Barnard, R., Bundesen, P. G., Rylatt, D. B. & Waters, M. J. (1984) Endocrinology (Baltimore) **115**, 1805–1813
12. Scatchard, G. (1949) Ann. N.Y. Acad. Sci **51**, 660–672
13. Barnard, R. & Waters, M. J. (1988) Biochem. J. **250**, 533–538
14. McPherson, G. A. (1985) J. Pharmacol. Methods **14**, 213–228
15. Barnard, R. & Waters, M. J. (1986) J. Receptor Res. **6**, 209–225
16. Woodward, M. P., Young, W. W. & Bloodgood, R. A. (1985) J. Immunol. Methods **78**, 143–153
17. Laemmli, U. K. (1970) Nature (London) **227**, 680–685
18. Shiu, R. P. C. & Friesen, H. G. (1974) J. Biol. Chem. **249**, 7902–7911
19. Okamura, H., Raguet, S., Bell, A., Gagnon, J. & Kelly, P. A. (1989) J. Biol. Chem. **264**, 5904–5911
20. Hughes, J. P. (1979) Endocrinology (Baltimore) **105**, 414–420
21. Hughes, J. P., Tokuhiro, E., Simpson, J. S. A. & Friesen, H. G. (1983) Endocrinology (Baltimore) **113**, 1904–1906
22. Thomas, H., Green, I. C., Wallis, M. & Aston, R. (1987) Biochem. J. **243**, 365–372
23. Husman, B., Haldosen, L., Andersson, G. & Gustafson, J. (1988) J. Biol. Chem. **263**, 3963–3970
24. Donner, J., Erikson, H. & Belfrage, P. (1986) FEBS Lett. **208**, 269–272
25. Mosier, M. D. & Lewis, U. J. (1982) Horm. Res. **14**, 440–441

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