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Purification of the 22000- and 20000-mol.wt. forms of human somatotropin and characterization of their binding to liver and mammary binding sites

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Quantitative data concerning the binding of 22000-mol.wt. human somatotropin and its 20000-mol.wt. variant are described using pregnant-rabbit liver and mammary-gland receptors. The purification and the complete chemical characterization of both human somatotropin and its 20000-mol.wt. variant is also presented. Contamination of the 20000-mol.wt.-variant preparation by 22000-mol.wt. hormone was found to be 0.5% by weight as measured in radioimmunoassay using monoclonal antibody. Labelling of human somatotropin and its 20000-mol.wt. variant using the Iodogen method is described as well as the characterization of the binding to pregnant-rabbit liver and mammary-gland receptor preparations. The maximum binding capacity of the ^{125}I-labelled human somatotropin was between 50 and 60% to liver particulate receptor, whereas that of the 20000-mol.wt. variant was 30%. The specificity of binding of both forms to rabbit hepatic and mammary-gland receptor was found to be similar for both proteins in the same system. The affinity constants and capacity were respectively $0.7 \times 10^{10}\,\mathrm{M}^{-1}$ and 815 fmol/mg of protein for human somatotropin and $0.6 \times 10^{10}\,\mathrm{M}^{-1}$ and 1.250 fmol/mg of protein for the 20000-mol.wt. variant. These data suggest that both proteins behave as partial agonists to the receptors studied.

The 20000-mol.wt. structural variant of human somatotropin was first isolated and chemically characterized by Lewis *et al.* (1978). The molecule was shown to have a 15-amino-acid deletion between residues 32 and 46 of the human somatotropin sequence (Lewis *et al.*, 1980). This 20000-mol.wt. variant possesses biological activities normally associated with the human somatotropin, except for the early insulin-like activity (Frigeri *et al.*, 1979).

It is well documented that the liver is a major site of action of somatotropin (McConaghey & Sledge, 1970), where it stimulates somatomedin production. Recently Spencer *et al.* (1981) demonstrated that the 20000-mol.wt. variant also stimulates somatomedin production *in vivo* by the liver.

Since Posner *et al.* (1974) have shown that the highest level of specific binding of ^{125}I-labelled somatotropin is found in the liver of late-pregnant rabbit, homogenates of this organ have been widely used as receptor preparations in radioreceptor assays for human somatotropin. However, some controversy exists as to whether these binding sites are somatogenic or lactogenic (Waters & Friesen, 1979; Tsushima *et al.*, 1980; Cadman & Wallis, 1981).

Recently, Sigel *et al.* (1981) published data on competitive binding between ^{125}I-labelled somatotropin and the 20000-mol.wt. variant. Nevertheless for binding to rabbit liver particulate receptors no results were presented concerning the binding of the iodinated variant itself.

The present study was designed to obtain quantitative binding data for both 22000- and 20000-mol.wt. somatotropin to membrane fractions obtained from liver and mammary glands of late-pregnant rabbit when studied under identical experimental conditions.

We also report in the present paper the purification and the chemical, biological and immunological characterization of both the 20000-mol.wt. variant and the 22000-mol.wt. human somatotropin. Indeed, a strict control of the quality and structural stability of both labelled and unlabelled proteins is essential for a correct interpretation of receptor binding data.

Materials and methods

Materials

Human somatotropin and its 20000-mol.wt. variant were prepared by the method of Lewis *et al.*

(1978) with the following minor modifications. First DEAE-Sephacel (Pharmacia Fine Chemicals) was used in place of DEAE-cellulose for all the anion-exchange chromatographies. Secondly, the fraction eluted at 4 mS from DEAE Sephacel was re-applied on the same exchanger and eluted with a linear gradient of 2–6 mS of NH_4HCO_3 and 6 M-urea.

Final gel filtration was performed on Ultrogel Aca 54 (Industrie Biologique Française) equilibrated in 0.05 M-sodium acetate (pH 5.0)/6 M-urea.

Bovine and porcine somatotropins were isolated by an extraction procedure described by Closset & Hennen (1974). The partially purified somatotropins adsorbed on CM-Sephadex C-25 (Pharmacia Fine Chemicals) were further purified on concanavalin A–Sepharose 4B (Pharmacia Fine Chemicals) equilibrated in 0.05 M-phosphate buffer, pH 7.4, to adsorb glycoprotein hormones. The somatotropins were then chromatographed on Sephadex G-100 (Pharmacia Fine Chemicals) equilibrated in 0.05 M-NH_4HCO_3.

As measured in radioreceptor assays the bovine somatotropin preparation exhibited an activity of 1.2 times that of the National Institute of Health bovine somatotropin preparation B18. Its contamination in prolactin was found to be 2% by weight as measured by specific radioimmunoassay. Human placental lactogen was from UCB Bioproducts, Brussels, Belgium. Its immunological potency amounted to 1 i.u./mg when measured against the Medical Research Council standard preparation 73/543. Human prolactin was a gift from Dr. H. Friesen. Its growth hormone contamination amounted to 0.2% by weight. Ovine prolactin (NIH P.S9) was a gift from the National Institute of Health. The (32–46)-peptide from the human somatotropin sequence was synthesized by UCB Bioproducts (Belgium). $Na^{125}I$ (carrier-free; IMS 30) was from The Radiochemical Centre, Amersham, Bucks., U.K. Iodogen 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluryl was purchased from Pierce Chemicals. New Zealand White pregnant rabbits were from our animal house. They were injected with 2 mg of bromocriptine/kg body wt. at 36, 24 and 12 h before being killed by the method of Djiane *et al.* (1977). Rabbits were killed 1–2 days before term.

Analytical methods

The amino acid composition was determined by the method of Spackman *et al.* (1958) using a model 121 Beckman Amino Acid Analyzer. Sodium dodecyl sulphate/polyacrylamide-gradient-gel electrophoresis was performed by the method of Laemmli (1970). Polyacrylamide-gel isoelectrofocusing was performed using PAGE plates (LKB Sweden) by the method of Vesterberg (1972). Peptide mapping of a tryptic digest of *S*-carboxymethylated somatotropin and its 20000-mol.wt. variant was performed on a Spectra Physics SP 800 B HPLC system equipped with variable-u.v. detector SP 8400 on a Polygosyl C18 column, which was eluted with a linear gradient of water/acetonitrile in water (3:2, v/v). Temperature was 40°C and flow rate was 0.7 ml/min.

Reduction and *S*-carboxymethylation of the proteins were performed by the method of Crestfield *et al.* (1963). Recrystallized iodoacetic acid (Janssen Pharmaceutica, Beerse, Belgium) was used for carboxymethylation. The amino acid sequence of residues 1–12 was determined by automated Edman degradation with a Beckman 890 C sequencer using 0.1 M-1,1′,1″,1‴-(1,2-ethanediyldinitrilo)-tetrakis-(propan-2-ol) (Quadrol) and single-amino acid, cleavage was performed by the method of Brauer *et al.* (1975). The initial amount of reduced and *S*-carboxymethylated 20000-mol.wt. variant was 0.05 μM. Phenylthiohydantoin amino acid derivatives were identified as described by Closset *et al.* (1978).

Bioassays

The growth-promoting activity was measured in the tibia bioassay by the method of Greenspan *et al.* (1949) in hypophysectomized female rats (Long–Evans strain). Proteins were injected subcutaneously at doses of 20 μg, 60 μg, 120 μg and 200 μg per rat.

Radioimmunoassays

The methods and the reagents used for the radioimmunoassay of human somatotropin, human lutropin, thyrotropin and prolactin were described by Vandalem *et al.* (1979) and Lequin *et al.* (1973).

Monoclonal antibody against 22000-mol.wt. human somatotropin

Balb/c mice were immunized by three subcutaneous injections of 10 μg of human somatotropin. Spleen cells (60×10^6) were fused with 13×10^6 cells of $X_{63}Ag_8$-653 non-secretory myeloma cell line kindly provided by Dr. B. Pau (Clin Midy, Montpellier, France) by the method of Köhler & Milstein (1975) with R.P.M.I. 1640 (GIBCO Biocult) as cloning medium supplemented with 15% calf serum, hypoxanthine (110 μM), aminopterin (0.4 μM) and thymidine (16 μM). Hybridomas were cloned by limited dilution. Clones producing anti-(human somatotropin) antibodies were screened by radioimmunoassays and enzymoimmunoassays by the method of Engvall (1980). Twenty clones produced anti-(human somatotropin) antibodies, ten of which have been growing in culture as established cell lines for 1 year and four of which continued to secrete monoclonal antibodies when injected intraperitoneally in Balb/c mice. The specificity of these mono-

clonal antibodies was studied by competitive radioimmunoassays using the hormones described in the Materials and methods section (Fig. 1).

Iodination of hormones

^{125}I-labelled human somatotropin, its 20000-mol.wt. variant and ovine prolactin were prepared by the Iodogen method of Fraker & Speck (1978). Iodogen (20μg) and Na^{125}I (1 mCi) were used to label 15μg of human somatotropin dissolved in 20μl of 0.5M-phosphate buffer adjusted to pH 7.4 for 5 min at room temperature. A similar procedure was used for the labelling of the human 20000-mol.wt. variant, except that 22μg of the protein was labelled for 10 min. Both tracers were purified on an Ultrogel Aca 54 column (1 cm × 100 cm) according to the conditions described in Fig. 2. In the case of ovine prolactin, 1 mCi of Na^{125}I was used for labelling 10μg of the hormone with 20 μg of Iodogen. Labelled hormone was purified by chromatography on Sephadex G-25 equilibrated in 0.05M-sodium phosphate/0.1% bovine serum albumin. Labelling and purification of bovine somatotropin was by the same method as that used for human somatotropin. Specific radioactivity of each tracer was measured by the self-displacement method in radioimmunoassays and radioreceptor assays as described by Ketelslegers *et al.* (1975). In the self-displacement experiments of ^{125}I-labelled somatotropin and of the 20000-mol.wt. variant, both curves were parallel. Fig. 3 shows the results of a typical experiment to estimate the specific radioactivity of ^{125}I-labelled human 20000-mol.wt. variant. Free and antibody-bound radioactivities were separated using a double-antibody solid-phase technique. After 4h, the tubes were centrifuged, the supernatants removed by aspiration and the pellets were counted for radioactivity (LKB model 1275; counting efficiency 75–80%).

Radioligand receptor assay

Binding and displacement studies were essentially performed as described by Tsushima & Friesen (1973) using a crude liver membrane preparation from late-pregnant rabbits. The incubation procedure was identical for both human somatotropin and the 20000-mol.wt. variant. The incubation time was 16h at room temperature. Each assay tube contained 20μl of 0.025M-Tris/HCl, pH 7.4, 0.1% bovine serum albumin, 10mM-$CaCl_2$, 10000 kallikrein units of trasylol/l, 100μl of membrane-bound receptor (120μg of soluble protein), 100μl of unlabelled hormone and 100μl of ^{125}I-labelled hormone (200000 c.p.m.). After addition of 1.5 ml of cold buffer to each tube, bound and free hormones were separated by centrifugation at 5200g for 20 min. The supernatants were removed by aspiration and the precipitates were counted for radioactivity. Binding that could not be displaced by an excess of 2μg of unlabelled highly purified hormone was considered to be non-specific and subtracted from total binding. The non-specific binding amounted to 4% and 9% for 22000-mol.wt. somatotropin and the 20000-mol.wt. variant respectively. A similar procedure was followed for the radioreceptor assay using rabbit mammary-gland receptors.

Scatchard analysis of binding data

Scatchard analysis (Scatchard, 1949) was performed using saturation and binding inhibition experiments in which a constant amount of receptor was incubated with increasing concentrations of radioactively labelled or unlabelled hormones. Maximum binding capacity (percentage of radioactively labelled hormone specifically bound to an excess of receptors) was used for correction. The hormone–receptor interaction was assumed to correspond to a reversible bimolecular reaction. Scatchard analysis and parallel-line assays were performed using the MLAB system described by Knott & Schrager (1972) running on a PDP-20 time-sharing Digital computer.

Results

Physicochemical, immunological and biological properties of human somatotropin and 200000-mol.wt.-variant preparations

On the basis of the physicochemical criteria described in the Materials and methods section both 22000- and 20000-mol.wt. proteins appeared fairly pure. Nevertheless careful assessment of the cross contamination of these two proteins is compulsory for a correct interpretation of receptor-binding data. Among the numerous monoclonal antibodies produced against the 22000-mol.wt. human somatotropin, only one ($K_1F_3E_6$) could distinguish between the 22000-mol.wt. hormone and its 20000-mol.wt. counterpart. As shown in Fig. 1, the contamination of the 20000-mol.wt. variant by the 22000-mol.wt. somatotropin amounted to 0.5% by weight when estimated in radioimmunoassay using ^{125}I-labelled human somatotropin as tracer. Contamination of both 22000- and 20000-mol.wt. proteins by prolactin and other glycoprotein hormones was always less than 0.01% by weight as measured in specific radioimmunoassays. The specific growth-promoting activity of the 20000-mol.wt.-variant preparation, which amounted to 1.8 i.u./mg in the tibia test (1.7–1.9 i.u./mg) was equipotent with that of human somatotropin, with a value of 1.85 i.u./mg (1.8–2.1 i.u./mg), if the 95% confidence limits are considered.

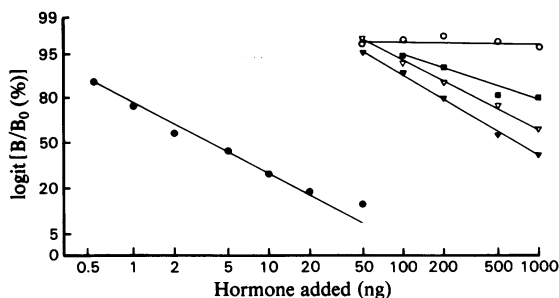

Fig. 1. *Radioimmunoassays for the 22000-mol.wt. somatotropin*

Antisera to the 22000-mol.wt. human somatotropin (Balb/c mice ascites fluid at the final dilution of 1:500000) were incubated with ^{125}I-labelled human somatotropin. For displacement the following unlabelled hormones were used: 22000-mol.wt. somatotropin, ●; 20000-mol.wt. variant of somatotropin, ▼; human choriogonadotropin, ○; human placental lactogen, ■; human prolactin, ▽. B is the ratio of specifically bound radioactivity (c.p.m.) to the total radioactivity (c.p.m.). B_0 is the ratio of specifically bound radioactivity (c.p.m.) to the total radioactivity (c.p.m.) in the absence of unlabelled hormone.

Characteristics of the tracers

Both 22000- and 20000-mol.wt. proteins were labelled by the Iodogen method and purified on Ultrogel Aca 54. The selected peak possessed an elution volume corresponding to that of the somatotropin monomer and similar in size to the native proteins (Fig. 2). The specific activity ranged between 60–70μCi/μg and 30–40μCi/μg for ^{125}I-labelled human somatotropin and the 20000-mol.wt. variant respectively (Fig. 3). More than 50% of the ^{125}I-labelled somatotropin was specifically bound to an excess of receptor preparation (600μg of protein), whereas about 30% of the labelled 20000-mol.wt. variant was bound using a similar hormone to receptor ratio. The specific radioactivity of the labelled bovine somatotropin amounted to 50μCi/μg, and its bindability using an excess of particulate liver receptor (600μg) was approx. 45%.

Study of binding reactions

Specific binding of both ^{125}I-labelled human somatotropin and its 20000-mol.wt. variant to liver particulate receptors was shown to be time- and temperature-dependent (Fig. 4). For both polypeptides, binding was faster at 37°C than at 25°C. Equilibrium was reached after 16h at 25°C, which was the temperature used routinely for radioreceptor assays, and after 3h when tested at 37°C. In all cases, non-specific binding reached its maximum after 10h incubations and remained constant thereafter.

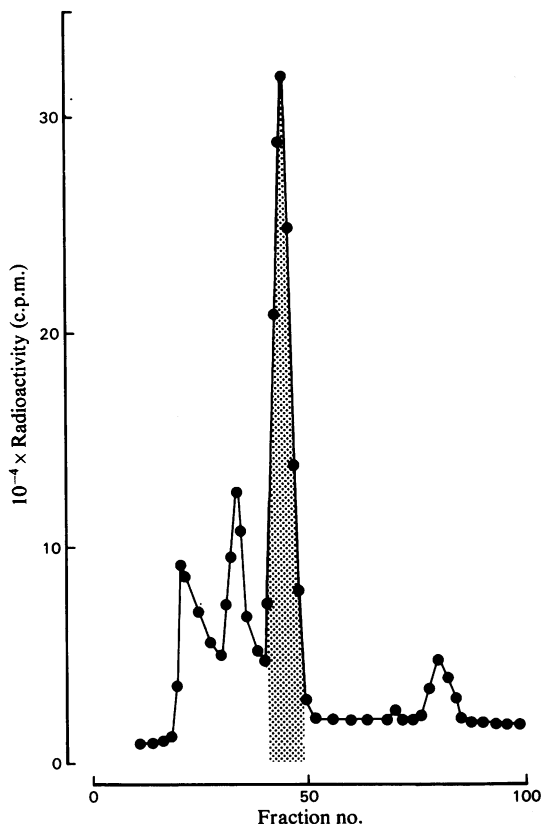

Fig. 2. *Chromatography of ^{125}I-labelled human 20000-mol.wt. variant on Ultrogel Aca 54*

The column (1cm × 100cm) was equilibrated in 0.05M-sodium phosphate, pH 7.4, containing 0.1% bovine serum albumin. Fraction volume was 1ml; a sample of 1mCi was applied in 1ml. The shaded area contains the ^{125}I-labelled 20000-mol.wt. variant monomer.

Binding specificity of ^{125}I-labelled human somatotropin and the 20000-mol.wt. variant for particulate liver receptors

Fig. 5(*a*) shows the displacement curves of ^{125}I-labelled human somatotropin by the human 20000-mol.wt. variant, somatotropin (32–46)-peptide, as well as by somatotropins, prolactins and placental lactogen from a variety of species. The data are very similar to those reported in the literature.

The human 20000-mol.wt. variant easily displaced ^{125}I-labelled humans somatotropin with a significantly different slope from that of unlabelled 22000-mol.wt. hormone. Bovine somatotropin was also capable of displacing ^{125}I-labelled human somatotropin in a manner comparable with that of the 20000-mol.wt. variant. Porcine somatotropin,

human and ovine prolactin, and human placental lactogen could also complete for the binding sites with the 22000-mol.wt. tracer but with a very low potency compared with that of human somatotropin. The (32–46)-peptide did not displace the ^{125}I-labelled somatotropin from the receptors.

The binding specificity for liver particulate receptor when using the labelled 20000-mol.wt. variant as a tracer was also investigated (Fig. 5*b*). The specificity of the interaction appeared similar to that found with the ^{125}I-labelled human somatotropin. Nevertheless, it has to be pointed out that using the labelled 20000-mol.wt. variant as tracer, the displacement curves obtained for both human somatotropin and the 20000-mol.wt. variant were parallel and the specific activity of the 22000-mol.wt. hormone appeared to be twice that of the 20000-mol.wt. variant. In contrast, Fig. 5(*c*) shows the displacement of ^{125}I-labelled bovine somatotropin by the hormones used in the former experiments. In this system, the potency of the various hormones tested agrees well with those measured in the system using human somatotropin and the 20000-mol.wt. variant as tracers.

Binding specificity of ^{125}I-labelled ovine prolactin for mammary-gland receptor

Fig. 5(*d*) shows the displacement of ^{125}I-labelled ovine prolactin from particulate mammary-gland receptors by ovine prolactin itself, the human somatotropin, the 20000-mol.wt. variant, and various hormones. Both the 22000-mol.wt. hormone and the 20000-mol.wt. variant, as well as ovine prolactin, give superimposable displacement curves.

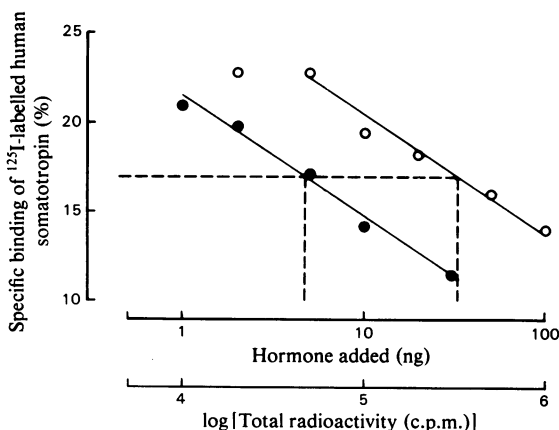

Fig. 3. *Determination of specific radioactivity of ^{125}I-labelled human 20000-mol.wt. variant*

^{125}I-labelled 20000-mol.wt. variant (approx. 35000 c.p.m.) was incubated with particulate liver receptor and increasing concentrations of labelled (○) or unlabelled (●) hormone. Specific binding was determined. Each value represents the mean of duplicate determinations.

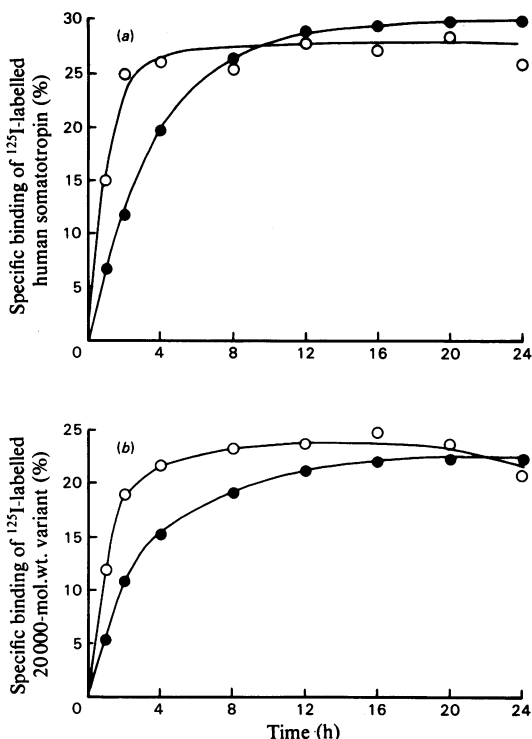

Fig. 4. *Effect of time and temperature on the binding of ^{125}I-labelled human somatotropin (a) and the 20000-mol-wt. variant (b) to liver particulate receptor*

^{125}I-labelled somatotropin (approx. 25000 c.p.m.) and ^{125}I-labelled 20000-mol.wt. variant (approx. 35000 c.p.m.) were incubated with liver receptor preparation (120 μg of protein) at 37°C (○) and 25°C (●) and specific binding was determined. Each value represents the mean of quadruplicate determinations.

Scatchard analysis of binding data

Fig. 6 shows Scatchard analysis of displacement curves. Similar results were also obtained from saturation curves. Reproducible data were recorded in a succession of similar experiments. The results using particulate liver receptor and ^{125}I-labelled human somatotropin (Fig. 6*a*) revealed a single class of binding sites for human somatotropin with a high affinity ($0.7 \times 10^{10}\,M^{-1}$) and a capacity of 815 fmol/mg of protein.

Using ^{125}I-labelled 20000-mol.wt. variant as tracer and the same liver particulate receptor (Fig. 6*b*) the affinity constant was found to be $0.6 \times 10^{10}\,M^{-1}$ with a slightly higher capacity of 1250 fmol/mg of protein.

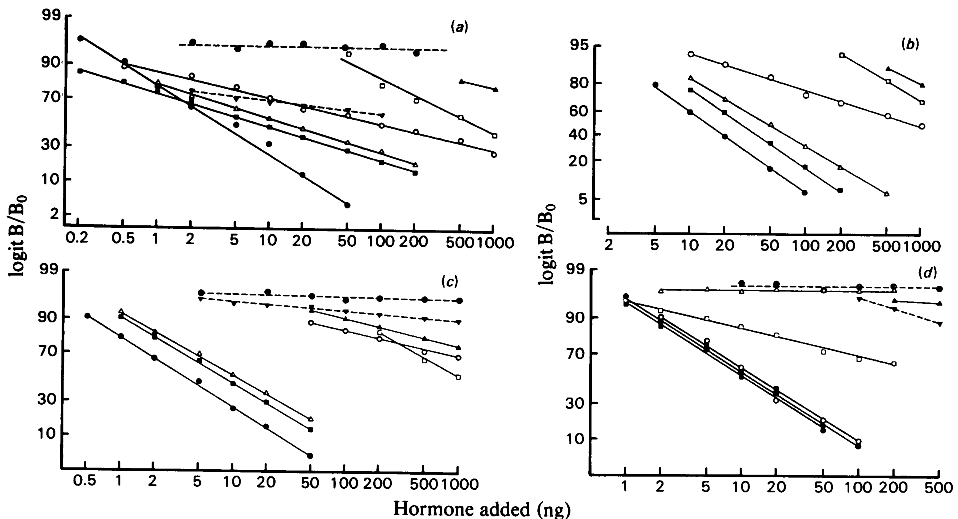

Fig. 5. *Specificity of human somatotropin, its 20000-mol.wt. variant and bovine somatotropin binding to liver and mammary gland receptors*

(*a*) ^{125}I-labelled human somatotropin (approx. 25000c.p.m.), (*b*) ^{125}I-labelled human 20000-mol.wt. variant (approx. 35000c.p.m.) and (*c*) ^{125}I-labelled bovine somatotropin (approx. 25000c.p.m.) was incubated with liver receptor preparation (120μg of protein) in the presence of increasing amounts of human somatotropin (●), 20000-mol.wt. variant (■), bovine somatotropin (△), porcine somatotropin (▲), ovine prolactin (○), human prolactin (□), human placental lactogen (▼) and (32–46)-peptide (○). (*d*) ^{125}I-labelled ovine prolactin (approx. 30000c.p.m.) was incubated with particulate mammary-gland receptor (200 μg of protein) in the presence of increasing amounts of the same hormones as in (*a*), (*b*) and (*c*). Each value represents the mean of duplicate determinations.

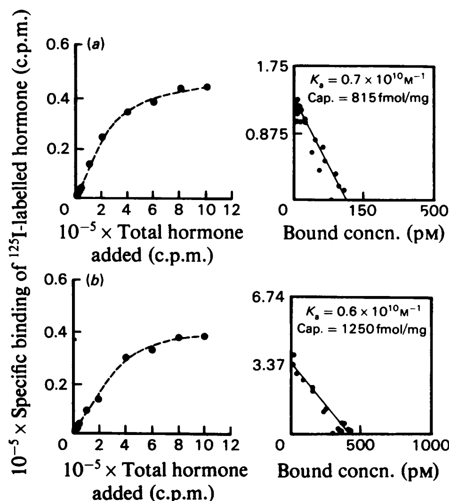

Fig. 6. *Scatchard analysis of saturation curves for* (*a*) ^{125}*I-labelled human somatotropin and* (*b*) ^{125}*I-labelled 20000-mol.wt. variant on liver particulate receptor*

Increasing amounts of ^{125}I-labelled proteins were incubated with appropriate amounts of receptor preparation (120μg of protein) and specific binding was determined. Scatchard analyses of the binding curves are shown on the right. Values of capacity (Cap.) are given in fmol/mg of protein and the units of K_a are M^{-1}.

Discussion

It has not been until recently that data concerning the binding receptors of the human 20000-mol.wt. variant began to appear in the literature. Sigel *et al.* (1981) have conducted competitive-binding experiments on pregnant-rabbit liver and mammary receptors using ^{125}I-labelled 22000-mol.wt. somatotropin only. Wohnlich & Moore (1982) have compared the binding of both 22000- and 20000-mol.wt. tracers on male and pregnant rabbit liver and female rat liver receptors on the basis of displacement experiments.

To obtain quantitative data (affinity and capacity of hormone binding) on the binding to pregnant-rabbit liver receptors we carried out a thorough investigation by Scatchard analysis of saturation and displacement curves for both 22000- and 20000-mol.wt. somatotropins. Special attention was paid to the quality of unlabelled hormones and to the tracers. The physicochemical and biological characterization of our 20000- and 22000-mol.wt. preparations provided analytical data essentially similar to those of Lewis *et al.* (1978). In addition contamination of the 20000-mol.wt.-variant preparation by the 22000-mol.wt. somatotropin was determined in radioimmunoassay using monoclonal

antibody raised against 22000-mol.wt. somatotropin. This was essential since such a contamination would lead to misinterpretation of binding properties. Similarly, the quality of the labelled hormones was assessed by gel filtration, autoradiography after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, bindability in the presence of an excess of receptors and determination of specific radioactivity by the self-displacement method.

Binding experiments were first performed on mammary-gland receptors using ^{125}I-labelled ovine prolactin as tracer, since a lactogenic activity of the 20000-mol.wt. variant in the mammary gland could not be presumed. In these experiments, the displacing potencies of the 20000- and 22000-mol.wt. human somatotropins were found to be identical. This fact is in agreement with the lactogenic potency of these proteins as measured in the pigeon crop-sac bioassay (Lewis *et al.*, 1978). The particular displacing potency of the 20000-mol.wt. variant in our experiments contrasts with the results of Sigel *et al.* (1981), who found a displacing activity of the 20000-mol.wt. variant four times less than that of human somatotropin. On the other hand, our results were in complete agreement with those of Shiu & Friesen (1974) and Cadman & Wallis (1981) with respect to the complete displacement of ^{125}I-labelled prolactin by an unlabelled somatogenic hormone (bovine somatotropin).

Binding characteristics (affinity and capacity) were studied using liver receptor preparations from pregnant rabbits. In order to characterize both somatogenic and lactogenic binding sites present in the liver preparation, we have compared the binding capacity of bovine and human somatotropins. It appeared that the binding is essentially due to the presence of somatogenic rather than lactogenic binding sites since the binding capacity (600 fmol/mg of protein) for bovine somatotropin was found to be 30% less than for human somatotropin (815 fmol/mg of protein). Moreover, ^{125}I-labelled human somatotropin and the 20000-mol.wt.-variant binding can be almost completely inhibited by unlabelled bovine somatotropin, whereas unlabelled prolactin is ten times less potent. These data contrast with those of Wohnlich & Moore (1982), who observed a lack of competition between bovine somatotropin and the ^{125}I-labelled 20000-mol.wt. variant as tracer.

Discrepancies between results could be explained by differences observed in the receptor bindability of ^{125}I-labelled somatotropin and the 20000-mol.wt. variant in the work of Wohnlich & Moore (1982) compared with our own work. Also, discrepancies could be due to the different physiological states of the rabbits selected to prepare liver receptor fractions. Indeed our experiments were performed on liver obtained from late-pregnant rabbits treated with bromocriptine 36 h before death, whereas Wohnlich & Moore (1982) and Sigel *et al.* (1981) used receptors prepared from pregnant rabbits. Such variations in the physiological states of the rabbits used for preparing liver receptors have been suggested by Kelly *et al.* (1974), who showed that there is an increase in hepatic binding sites during pregnancy essentially due to an increase in somatogenic rather than lactogenic receptors. On the other hand, it should be remembered that the displacement curves obtained with both proteins and ^{125}I-labelled 22000-mol.wt. somatotropin as tracer were non-parallel in our experiments. This is certainly not enough evidence to conclude that the binding sites are different for the 22000- and 20000-mol.wt. somatotropins. Non-parallelism in displacement experiments could theoretically be due to numerous factors. Among others, conformational differences of both labelled and unlabelled hormones could possibly take place during the period of time used to reach a steady state in binding experiments. That such a process affects one of these proteins more than the other could explain the observed differences. Indeed it has been shown that the 20000-mol.wt. somatotropin is prone to dimerization and is found in greater proportion in the dimeric fraction of somatotropin during the purification of clinical-grade somatotropin. Another possible explanation for the non-parallelism is that the deleted sequence of residues 32–46 in the 20000-mol.wt. variant could be of primary or secondary importance in the binding of the human somatotropin to these liver receptors as suggested to Wohnlich & Moore (1982).

Although the bulk of binding sites of human somatotropin in the hepatic receptor preparation used in our study appear to be due to the presence of somatogenic and lactogenic receptors, Scatchard analysis of saturation and displacement curves for both 22000-mol.wt. somatotropin and the 20000-mol.wt. variant suggested a single class of saturable binding sites with almost identifical affinity constant and similar number of binding sites. The similarity in the affinity constants agrees well with the identical growth-promoting activities *in vivo* of these molecules as described by Lewis *et al.* (1978) and confirmed in the present paper.

Similarity in the affinity and the capacity of liver receptors for the 22000- and 20000-mol.wt. variants and in their specificities, as studied with various somatogenic and lactogenic hormones, supports the notion that the 20000- and 22000-mol.wt. human somatotropins behave essentially as partial agonists at this receptor level.

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