Lactogenic and somatogenic binding sites in intact and detergent-solubilized membrane preparations of female rat liver

Lars-A. HALDOSÉN* and Jan-Å. GUSTAFSSON

Department of Medical Nutrition, Karolinska Institute, Huddinge University Hospital F69, S-141 86 Huddinge, Sweden

The presence of lactogenic and somatogenic binding sites in intact microsomal membranes and in detergent-solubilized microsomal membrane preparations of female rat liver has been studied by affinity cross-linking-SDS/polyacrylamide-gel electrophoresis. In microsomal membrane preparations an M_r 40000 lactogenic binder is present which is not disulphide-linked to another protein. Triton X-100 solubilization of membranes results in the appearance of three lactogenic ^{125}I -human growth hormone (^{125}I -hGH) binders with M_r values of 87000, 40000 and 35000, and one somatogenic ^{125}I -hGH binder with M_r 32000. Treatment of rats with oestrogen increased the amount of lactogenic and somatogenic binding species in liver. The lactogenic binding sites are present as one entity in Triton X-100-solubilized preparations, clearly separated from the somatogenic binder as analysed by gel chromatography. Furthermore, ^{125}I -hGH interacts with an M_r 95000 somatogenic binder in membrane preparations to which the hormone can be cross-linked only following Triton X-100 solubilization.

INTRODUCTION

The presence of binding sites for both prolactin and growth hormone in rat liver is well documented (Kelly *et al.*, 1974; Ranke *et al.*, 1976; Rae-Venter & Dao, 1982). Hepatic lactogenic receptors are present in both sexes, but at a much higher concentration in female liver (Kelly *et al.*, 1974). A clear-cut role for prolactin and the lactogenic receptor in regulating liver function has not yet been demonstrated, although reports have appeared showing prolactin-induced responses in liver (for references see Haldosén & Gustafsson, 1987).

Hepatic growth hormone receptors are present at nearly equal concentration in both sexes (Husman *et al.*, 1985). The sexually differentiated contents in rat liver of steroid-metabolizing cytochrome *P*-450 enzymes and prolactin receptors are regulated by the sexually differentiated secretory rhythm of growth hormone (Mode *et al.*, 1982; Norstedt, 1982; Baxter & Zaltsman, 1984).

Structural data for the prolactin and growth hormone binding entities in rat liver has been obtained by the use of different techniques, such as gel filtration and sucrose density-gradient centrifugation in the case of prolactin receptor and affinity cross-linking-SDS/polyacrylamide-gel electrophoresis (SDS/PAGE) for both types of receptors. The latter method has revealed lactogenic and somatogenic binding species with M_r values ranging from 28000 to 87000 and 110000 to 290000, respectively (Borst & Sayare, 1982; Hughes *et al.*, 1983; Yamada & Donner, 1984; Bonifacino & Dufau, 1985; Borst *et al.*, 1985; Haldosén & Gustafsson, 1987). In contrast with affinity cross-linking of lactogenic hormone to binding species, affinity cross-linking of somatogenic hormone to binding species has been reported to occur only when whole hepatocytes have been used but not in membrane or detergent-solubilized membrane preparations of rat

liver (Donner, 1983). By hydrodynamic characterization of detergent-solubilized rat liver membrane preparations, molecular masses of 73000, 78000 and 92000 for the lactogenic receptor could be calculated (Jaffe, 1982; Rae-Venter & Dao, 1983; Haldosén & Gustafsson, 1987).

In order to study the mechanisms of regulation of sexually differentiated properties (e.g. steroid-metabolizing cytochrome *P*-450 enzymes and prolactin receptor) in rat liver at a molecular level, a knowledge of the structure of the prolactin and growth hormone receptors in liver is needed. The aim of this study was to identify and determine some basal structural parameters of specific lactogenic and somatogenic binding entities in intact microsomal membrane and detergent-solubilized microsomal membrane preparations of rat liver.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats from Anticimex (Stockholm, Sweden), 8–10 weeks of age, were used throughout the study. In general, rats were injected subcutaneously with a depot dose of 1 mg of 17 β -oestradiol-valerate (Progynon-Depot, Schering AG, Berlin, Germany) 1 week before the start of the experiments. In some experiments, as indicated in the text, rats were untreated.

Chemicals

Triton X-100, bovine serum albumin and β -mercaptoethanol were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Prepacked Sephadex G-25 columns (PD-10), Sephadex G-75, Sephacryl S-400, Blue Dextran 2000 and ferritin (horse spleen), were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Dimethyl sulphoxide was obtained from Merck (Darmstadt, Germany). Iodogen (1,3,4,6-tetrachloro-3 α ,6 α -diphenyl-

Abbreviations used: hGH, human growth hormone; bGH, bovine growth hormone; rGH, rat growth hormone; oPRL, ovine prolactin; rPRL, rat prolactin; PAGE, polyacrylamide-gel electrophoresis; DSS, disuccinimidyl suberate.

* To whom all correspondence should be addressed.

glycouril), and disuccinimidylsuberate (DSS) were purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.). SDS was obtained from Kebo-Lab AB (Stockholm, Sweden). Na^{125}I (sp. activity 1 mCi/10 μl) and $^3\text{H}_2\text{O}$ (sp. activity 5 mCi/ml) were obtained from The Radiochemical Centre (Amersham, England, U.K.) and Fluka AG (Buchs, Switzerland) respectively. Reagents for SDS/PAGE were obtained from Bio-Rad (Richmond, CA, U.S.A.). All other chemicals were analytical grade products from either Sigma or Merck.

Hormones

Human growth hormone (hGH) (Crescormon; 2 i.u./mg) and biosynthetic methionyl-somatotropin (Somatonorm; 2 i.u./mg) were kind gifts from AB Kabi (Stockholm, Sweden). Ovine prolactin (oPRL) (NIADDK-oPRL-16), bovine growth hormone (bGH) (NIH-GH-B-14), rat growth hormone (rGH) (NIAMDD-rGH-B-6) and rat prolactin (rPRL) (NIAMDD-rPRL-B-3) were obtained from the Hormone Distribution Program (National Institute of Arthritis, Metabolism and Digestive Diseases, NIH Bethesda, MD, U.S.A.).

Iodination of hormones

^{125}I -hGH and ^{125}I -oPRL were prepared by the Iodogen method (Fraker & Speck, 1978). For a detailed description see Haldosén & Gustafsson (1987). A specific radioactivity of 30–40 $\mu\text{Ci}/\mu\text{g}$ of hormone was repeatedly obtained by using this method.

Preparation of hepatic microsomal membranes

Livers were perfused with a solution containing 150 mM-KCl and 10 mM-EDTA, pH 7.5, (EK-buffer) and removed into ice-cold EK-buffer. A 20% (w/v) homogenate in EK-buffer was prepared. After centrifugation for 15 min at 15000 g at 4 °C, the supernatant was recentrifuged at 105000 g for 80 min at 4 °C. The resulting pellet was resuspended in a Tris buffer (50 mM, pH 7.5). This microsomal membrane fraction was either frozen at –70 °C, used directly for detergent-solubilization, or used directly in binding assays.

Solubilization of hepatic membranes

Triton X-100 [final concn. 1% (v/v)] was added to microsomal membranes (protein concn. 5 mg/ml). After stirring for 1 h at room temperature the suspension was centrifuged at 105000 g for 70 min at 4 °C. The clear supernatant (3–4 mg/ml) was removed and used immediately or stored frozen at –70 °C.

Binding of iodinated hormone to membranes or solubilized preparation

Portions of membranes or solubilized preparation (0.3 ml, 250–450 μg of protein) were incubated with iodinated hormone (1×10^5 – 4×10^5 c.p.m.) for 18–20 h at 20 °C in the absence or presence of varying concentrations of different unlabelled hormones.

Gel-permeation chromatography

Gel-permeation chromatography of either ^{125}I -hGH-incubated, or ^{125}I -hGH-incubated and cross-linked Triton X-100-solubilized, preparations was performed with a Sephacryl S-400 column (2.5 cm $\times$ 90 cm), equilibrated and eluted with Tris buffer containing 0.1% (v/v) Triton X-100 (Tris/Triton buffer). The column was calibrated with ferritin and bovine serum albumin. The

void volume (V_0) was determined by using Blue Dextran 2000 and the total volume (V_t) by using $^3\text{H}_2\text{O}$. The calculations of Stokes radii were performed according to Porath (1963). The eluate fractions from chromatography of ^{125}I -hGH-incubated solubilized sample were cross-linked with 0.5 mM-DSS and subjected to SDS/PAGE. The eluate fractions from ^{125}I -hGH-incubated and cross-linked solubilized preparations were used directly for SDS/PAGE.

Cross-linking of iodinated hormone to membranes and to solubilized preparations

DSS in dimethyl sulphoxide was added to labelled membrane or solubilized preparations (final concn. 0.5 mM). In some experiments the final concentration of DSS was varied between 0.1 and 1.5 mM. These experiments indicated that a final concentration of 0.5 mM-DSS gave a low radioactive background, good resolution of labelled bands and a low amount of cross-linked material at higher M_r values (results not shown). After 15 min at 4 °C, 0.15 vol. of ice-cold 1 M-Tris (pH 7.5) was added to the samples to terminate the reaction.

Electrophoresis and autoradiography

Cross-linked samples were mixed with 0.6 vol. of SDS solubilizing buffer (Haldosén & Gustafsson, 1987) and heated at 100 °C for 2 min. SDS/PAGE was performed on 1.5 or 3 mm thick 7.5% polyacrylamide gels according to the method of Laemmli (1970). Autoradiography was carried out on dried gels in cassettes with Fuji RX X-ray film using a Dupont Cronex Lightning-Plus enhancing screen. The following ^{14}C -labelled marker proteins were used: phosphorylase *b* (94000), albumin (67000), catalase (60000) and ovalbumin (43000).

RESULTS

hGH (Crescormon) and biosynthetic methionyl-somatotropin (Somatonorm) have been used for labelling lactogenic and somatogenic binding sites. No quantitative or qualitative differences were observed between these hormones. hGH has been shown to bind to growth hormone and prolactin receptors in rat liver (Ranke *et al.*, 1976).

The microsomal membrane preparation used in this study has earlier been characterized by enzyme-marker analysis (Andersson *et al.*, 1978). It is slightly enriched in microsomes and Golgi vesicles and slightly depleted of lysosomes and plasma membranes as compared with a total particulate fraction of rat liver. The presence of mitochondria is greatly reduced.

The hormonal specificity of ^{125}I -hGH binding in Triton X-100-solubilized preparation was tested by adding to the incubations a 50- or 500-fold excess of different unlabelled hormones (Fig. 1). bGH, having exclusively growth-promoting effects in rodent (Kleinberg & Frantz, 1971), and rPRL were used to distinguish somatogenic and lactogenic binding of hGH, respectively. hGH inhibited the appearance of the bands at M_r 109000, 62000, 57000 and 54000. rPRL, especially at the higher concentration tested, prevented the appearance of the bands at M_r 109000, 62000 and 57000. bGH reduced the band at M_r 54000 only at the higher concentration.

The specificity of cross-linking of ^{125}I -hGH to microsomal membranes was tested under the same conditions

Fig. 1. Specificity of cross-linking of ^{125}I -hGH to Triton X-100-solubilized membrane preparation

Triton X-100-solubilized preparation (250 μg of protein) was incubated with ^{125}I -hGH (2.5×10^5 c.p.m.) in the presence or absence of a 50- or 500-fold excess ($\times$) of unlabelled hormone at 20 $^\circ\text{C}$ for 18 h. Cross-linking was performed with 0.5 mM-DSS at 4 $^\circ\text{C}$ for 15 min. Portions were taken for SDS/PAGE, performed on 3 mm thick 7.5% polyacrylamide gel under reducing conditions. Autoradiogram of dried gel is shown. The positions of the ^{14}C -labelled molecular mass markers are shown on the left of the autoradiogram and the major labelled species on the right.

Fig. 2. Effect of oestradiol treatment of female rats on the quantitative and qualitative autoradiographic appearance of cross-linked ^{125}I -hGH receptors

Microsomal membranes (lanes A and B), and Triton X-100-solubilized membranes (lanes C and D) were prepared from either untreated or oestradiol (E_2)-treated female rats as described in the Materials and methods section. These preparations (250 μg of protein) were incubated with ^{125}I -hGH (2.5×10^5 c.p.m.) at 20 $^\circ\text{C}$ for 18 h. Cross-linking was performed with 0.5 mM-DSS at 4 $^\circ\text{C}$ for 15 min. Portions were taken for SDS/PAGE-autoradiography, carried out as described in the legend to Fig. 1.

as mentioned above for Triton X-100-solubilized membranes (results not shown). The appearance of an M_r 62 000 band was inhibited by hGH and rPRL. bGH did not reduce the radioactive band. When ^{125}I -hGH-incubated and cross-linked samples of the micro-

Fig. 3. Specificity of cross-linking ^{125}I -hGH to Triton X-100-solubilized incubated microsomal membrane preparation

Microsomal membranes (400 μg of protein) were incubated with ^{125}I -hGH (2×10^5 c.p.m.) in the absence or presence of unlabelled hormone at 20 $^\circ\text{C}$ for 18 h. The pellet obtained after centrifugation for 20 min at 10000 g in an Eppendorf centrifuge 5413 was dissolved in phosphate-buffered saline (137 mM-NaCl/1.5 mM- KH_2PO_4 /16 mM- Na_2HPO_4 /3 mM-KCl, pH 7.4) containing 1% (v/v) Triton X-100. After 1 h, cross-linking was performed with 0.5 mM-DSS at 4 $^\circ\text{C}$ for 15 min. Portions were taken to SDS/PAGE-autoradiography, carried out as described in the legend to Fig. 1.

somal membrane preparation were run on SDS/PAGE in the absence or presence of reductant, a specific radioactive band at M_r 62 000 was seen under both conditions, but no additional specific radioactive band at a higher molecular mass was seen in the unreduced sample (results not shown). SDS autoradiography of ^{125}I -oPRL-incubated and cross-linked microsomal membranes showed the presence of a specific band at an approximate M_r value of 62 000 (results not shown).

All of the above-presented cross-linking studies have been performed with preparations from oestrogen-treated female rats. To examine the effect of oestrogen treatment on the autoradiographic intensities of the individual specific bands, preparations were obtained from livers of treated and untreated female rats. These preparations, containing equal amounts of protein, were incubated with equal amounts of ^{125}I -hGH and subjected to SDS/PAGE (Fig. 2). The intensity of the band at M_r 62 000 was markedly increased by oestrogen treatment in the microsomal membrane preparation (Fig. 2, lanes A and B). This was also the case with the bands at M_r 109 000, 62 000, 57 000 and 54 000 in the Triton X-100-solubilized preparation (Fig. 2, lanes C and D).

When ^{125}I -hGH-incubated membrane preparation was Triton X-100 solubilized after removal of unbound hormone by centrifugation, cross-linked specific autoradiographic bands at M_r 117 000, 109 000 and 62 000 were seen (Fig. 3). hGH inhibited the appearance of all three bands. rPRL, especially at the higher concentration tested, inhibited the appearance of the bands at M_r 109 000 and 62 000. rGH and bGH, at both concentrations tested, only prevented the appearance of the M_r 117 000 band.

Fig. 4. Sephacryl S-400 chromatography of incubated and DSS-treated Triton X-100-solubilized membrane preparation with subsequent SDS/PAGE of eluate

Triton X-100-solubilized preparation (20 ml, 80 mg of protein) was incubated with ^{125}I -hGH (23×10^8 c.p.m.) at 20°C for 18 h. Cross-linking was performed with 0.5 mM-DSS at 4°C for 15 min. The incubated sample was applied to a Sephacryl S-400 column (2.5 cm $\times$ 90 cm) equilibrated in Tris/Triton X-100 buffer and eluted with the same buffer at a flow-rate of approx. 23 ml/h. Fractions (10 ml) were collected and assayed for radioactivity. The column was calibrated with Blue Dextran (V_0), $^3\text{H}_2\text{O}$ (V_t) and the following standard proteins: *a*, ferritin (6.1 nm) and *b*, albumin (3.59 nm). Portions of the eluted fractions were taken for analysis with SDS/PAGE-autoradiography, carried out as described in the legend to Fig. 1. In the upper part of the Figure, the autoradiograms of dried gels are shown. It should be noted that the autoradiogram is not aligned with the radioactivity profile of the chromatography eluate.

Chromatography of the ^{125}I -hGH-incubated Triton X-100-solubilized preparation revealed a major peak at 6.1 nm and a minor peak at the position of free ^{125}I -hGH (results not shown). Cross-linking and SDS/PAGE of each individual fraction revealed the autoradiographic appearance of bands at M_r 109 000, 62 000 and 57 000 only from the fractions representing the 6.1 nm peak. No band at M_r 54 000 was found in any of the fractions (results not shown). Chromatography of the ^{125}I -hGH-incubated and cross-linked Triton X-100-solubilized preparation revealed one major radioactive peak at 6.1 nm and one minor peak at the position of free ^{125}I -hGH (Fig. 4, lower panel). When each of the eluted fractions was analysed by SDS/PAGE, the fractions representing the 6.1 nm peak were shown to contain binding species

Fig. 5. A compilation of values for molecular masses and Stokes radii for ^{125}I -hGH specific binders in different preparations of rat liver as obtained by different methods

The abbreviations used in this Figure are: DSS, affinity cross-linking with DSS; G.F., gel filtration on Sephacryl S-400; Triton X-100, solubilization of microsomal membrane proteins with Triton X-100; (L), lactogenic binding activity; (S), somatogenic binding activity.

that gave rise to M_r 109 000, 62 000 and 57 000 bands (Fig. 4, upper part, fractions 30–35). Fractions eluting in the 3.6 nm region contained material which gave rise to radioactive bands of M_r 88 000, 66 000, 62 000 and 54 000 (Fig. 4, upper part, fractions 37 and 38). In nearly all fractions containing radioactivity an autoradiographic band at M_r 44 000 was seen. This probably represents a cross-linked dimer of ^{125}I -hGH. It is conceivable that the M_r 88 000 and 66 000 bands also represent aggregates of ^{125}I -hGH.

DISCUSSION

Cross-linking of ^{125}I -hGH to microsomal membrane proteins revealed one specific lactogenic band at M_r 62 000. This was also the case when ^{125}I -hGH was substituted with ^{125}I -oPRL. Assuming a 1:1 binding ratio and an M_r of 22 000 for hGH, the M_r of the unoccupied binder would be 40 000 (Fig. 5). This is well in accordance with earlier published studies on female rat liver membranes (Borst & Sayare, 1982; Hughes *et al.*, 1983; Borst *et al.*, 1985).

When running cross-linked microsomal preparation on SDS/PAGE in the absence of reductant only the band at M_r 62 000 was seen. Using *N*-ethylmaleimide during the preparation and incubation of membranes from rat ovaries, Bonifacino & Dufau (1984) were able to show a lactogenic band in cross-linking experiments with an M_r of 100 000 in addition to a band at M_r 60 000. The rationale behind the use of *N*-ethylmaleimide was that this agent might inhibit the cleavage of the disulphide-linked form of the lactogenic receptor present in the membranes. We performed this type of experiment with microsomal membranes, but could not detect any other specific band than the M_r 62 000 band (results not shown). Thus, we conclude that, in female rat liver microsomal membranes, the lactogenic hormone binding species accessible for covalent binding has an M_r of 40 000 and is not disulphide-linked to another protein.

Cross-linked Triton X-100-solubilized membrane pre-

paration exhibited three bands with M_r values of 109 000, 57 000 and 54 000 in addition to the band at M_r 62 000 present in this and in the cross-linked membrane preparation. The bands with M_r values of 109 000 and 57 000 had lactogenic binding specificity whereas the third band with an M_r of 54 000 displayed somatogenic binding specificity. Occasionally, a lactogenic band with an M_r of 187 000 was also seen. The M_r values of the unoccupied lactogenic binders would be 165 000, 87 000, 40 000 and 35 000 (Fig. 5). The unoccupied somatogenic binder would have an M_r of 32 000 (Fig. 5). Quite similar findings have recently been presented by Bonifacino & Dufau (1985). These authors described three ^{125}I -hGH-binders in cross-linked Triton X-100-solubilized rat liver membranes with M_r values of 83 000, 36 000 and 32 000. They did not present any specificity data on these binders and therefore a direct comparison of their results with ours cannot be made regarding lactogenic and somatogenic binding properties of these binders.

It is known that oestrogen treatment of intact female rats increases the capacity of their livers to bind lactogenic hormone (Posner *et al.*, 1974). In the present study it was found that the intensity of both the lactogenic and the somatogenic bands was increased by oestrogen treatment, indicating an increased amount of both of these types of binders. The effect of oestrogen might be explained by an effect of the steroid on the secretion of growth hormone. It is known that the pulsatile secretory pattern of growth hormone is sexually differentiated (Mode *et al.*, 1982). In male rats the pulses occur at regular 3–4 h intervals and in between plasma GH levels are low or undetectable. In females, the pulses are low and base-line plasma GH levels are higher than in males. A continuous administration of growth hormone, in contrast with intermittent administration, which resembles the female and the male secretory pattern of growth hormone, respectively, to hypophysectomized female and male rats has been shown to induce lactogenic binding sites in liver (Norstedt *et al.*, 1981; Norstedt, 1982). The same type of administration has also been shown to induce both lactogenic and somatogenic binding sites in livers of intact and hypophysectomized female rats (Baxter & Zaltsman, 1984). It is known that oestrogen-treatment of male rats and gonadectomized female rats changes the serum pattern of GH to a pattern similar to that found in intact female rats (Mode *et al.*, 1982). It is not known what effect oestrogen treatment has on intact female adult rats with respect to growth hormone secretory pattern. It might be that the hormone is secreted in a continuous fashion but that the base-line level is set higher and that this is responsible for an induction of lactogenic and somatogenic binding sites.

When, after removal of unbound hormone, membrane preparation was Triton X-100-solubilized and thereafter cross-linked, an M_r 87 000 lactogenic binder appeared on the autoradiogram in addition to the M_r 40 000 binder (Fig. 5). No M_r 35 000 lactogenic binder was seen. This indicates that the M_r 87 000 binder interacts with ^{125}I -hGH but cross-linking only occurs after Triton X-100-solubilization. It also shows that the M_r 35 000 binder is not exposed for binding in the membrane preparation.

The appearance of a somatogenic binder with an M_r of 32 000 in the detergent-solubilized preparation is interesting. No somatogenic bands were detected in cross-linked membranes. By using female rat hepatocytes Yamada & Donner (1984) were able to detect three

somatogenic binders with M_r values of 280 000, 200 000 and 100 000. No specific bands were found when isolated membranes were used. In a study using intact rat adipocytes in cross-linking experiments, Gorin & Goodman (1984) detected four somatogenic binders with M_r values of 290 000, 220 000, 110 000 and 34 000. Interestingly, when incubated microsomal membrane preparation was Triton X-100-solubilized after removal of unbound hormone, a cross-linked somatogenic binder with M_r 95 000 was detected but not the M_r 32 000 binder (Fig. 5). This indicates that ^{125}I -hGH must interact with the M_r 95 000 binder in membrane preparation but cross-linking does not occur. The reason for the appearance of the M_r 95 000 binder could be that it is stabilized by hormone during solubilization and thereafter can be cross-linked with ^{125}I -hGH. Another explanation could be that after Triton X-100 solubilization of incubated membranes we did not centrifuge to remove unsolubilized material as we ordinarily do when preparing Triton X-100-solubilized membranes. The M_r 95 000 binder could be present in this unsolubilized material.

Analysis of incubated Triton X-100-solubilized membrane preparation with gel chromatography-SDS/PAGE revealed that the somatogenic binder and lactogenic binders are separate entities in detergent-solubilized state (Fig. 5). Co-chromatography of the lactogenic binders shows that they are present as one entity in detergent-solubilized state. By two-dimensional gel electrophoresis of incubated and cross-linked rat liver Triton X-100-solubilized membrane preparations we have shown that M_r 40 000 and 35 000 binding species are released from M_r 87 000 species (Haldosén & Gustafsson, 1987). The need for cross-linking the incubated preparation before chromatography in order to detect the somatogenic M_r 32 000 binder could either be explained by low-affinity binding between hormone and binder or degradation of the uncross-linked binder during chromatography and the subsequent release of iodinated hormone. The finding of an M_r 40 000 binder when cross-linking before chromatography and its co-chromatography with the somatogenic binder is interesting. From the results presented it cannot be concluded if this M_r 40 000 binder represents a lactogenic binder.

The present study has identified lactogenic and somatogenic binders in different preparations of female rat liver. In microsomal membrane preparation an M_r 40 000 lactogenic binder is present which is not disulphide-linked to another protein. Triton X-100 solubilization of membranes results in the appearance of three lactogenic binders and one somatogenic binder with M_r values of 87 000, 40 000, 35 000 and 32 000 respectively. Treatment of rats with oestrogen increased the amount of lactogenic and somatogenic binding species in liver. The lactogenic binders are present as one entity in Triton X-100-solubilized preparation, clearly separated from the somatogenic binder as analysed by gel chromatography. Furthermore, hGH interacts with an M_r 95 000 somatogenic binder in membrane preparation to which the hormone can be cross-linked only following Triton X-100 solubilization.

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