Characterization of Prolactin Binding by Membrane Preparations from Rat Liver

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Binding sites for prolactin were identified in a plasma-membrane-enriched fraction isolated from livers of mature female rats. ^{125}I -labelled sheep prolactin prepared by the lactoperoxidase procedure retained the same molecular integrity and binding affinity as the native hormone at physiological pH. The receptors bound prolactin from different species, whereas non-lactogenic hormones were not bound. The binding of ^{125}I -labelled sheep prolactin was activated equally by bivalent and univalent cations, bivalent cations exerting their maximal effect at much lower concentrations. The association of ^{125}I -labelled sheep prolactin with the receptor was a time- and temperature-dependent process. Partial dissociation was detected. The binding of ^{125}I -labelled sheep prolactin was strongly influenced by pH, with an optimum observed at pH 6.5. Receptor activity was destroyed by Pronase and phospholipase C, whereas neuraminidase increased binding. Treatment of the membranes by ribonuclease and deoxyribonuclease did not affect the binding. Binding of ^{125}I -labelled sheep prolactin was inhibited by *p*-chloromercuribenzoic acid, dithiothreitol and by brief exposure to high temperatures. Scatchard analysis of the binding of ^{125}I -labelled sheep prolactin to receptors indicated that prolactin has a high affinity for its receptor. Binding of prolactin to liver membranes showed some properties different from those observed with mammary cells. Binding by these tissues differed in pH optimum, in effects of ions, and in response to neuraminidase.

The characteristics of prolactin binding to rabbit mammary tissue have been studied in considerable detail (Shiu & Friesen, 1974a), and subsequently a prolactin receptor was solubilized and isolated from that tissue (Shiu & Friesen, 1974b). Antibodies to the purified receptor have been used to show that inhibiting this interaction of the hormone with the receptor will prevent a physiological response of mammary-gland cells to prolactin (Shiu & Friesen, 1976). It has also been shown that prolactin binds to other tissues in several species in addition to mammary gland, including liver, kidney, adrenal and ovary (Turkington & Frantz, 1972; Kelly *et al.*, 1974; Posner *et al.*, 1974; Shiu & Friesen, 1974a; Parke & Forsyth, 1975), as well as seminal vesicles, epididymis and prostate in the male reproductive system (Turkington & Frantz, 1972; Aragona & Friesen, 1975) and rat uterus (Williams *et al.*, 1978). In most of these tissues, the characteristics of the prolactin-binding reaction have not been investigated, and it is not known if all tissues that bind prolactin do so in the same manner as mammary cells. It would seem unlikely that all of the above-mentioned tissues would respond simultaneously to prolactin, and it seems logical that there should be some mechanism

for allowing for a selective response among the several tissues that bind the hormone. A partial solution to the selectivity problem could be variation in number, or affinity, of prolactin receptors. Several studies have indicated that the extent of prolactin binding by animal tissues can be modulated by hormones. These include liver, in which prolactin apparently regulates the number of its own receptors (Posner, 1975; Posner *et al.*, 1975; Kelly *et al.*, 1975; Knazek *et al.*, 1977). Effects of other hormonal environments on prolactin binding by liver (Gelato *et al.*, 1975; Aragona *et al.*, 1976) and by kidney and adrenal (Marshall *et al.*, 1978) have also been reported. Since prolactin apparently regulates the abundance of its own receptors, this might not be a suitable mechanism for discriminating among several tissues that could bind the hormone and respond to it. Therefore we have examined the characteristics of prolactin binding to rat liver membrane preparations *in vitro*. Liver seemed an appropriate tissue for the comparative studies. A precise physiological role for prolactin in regulating liver function has not yet been defined. However, recent work suggests that prolactin has significant effects on rat liver. The stimulation of RNA synthesis (Chen *et al.*, 1972), the increase in activity of ornithine decarboxylase (EC 4.1.1.17) and thymidine kinase (EC 2.7.1.75) in the

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liver of prolactin-treated animals (Richards, 1975; Thomson & Richards, 1978) and the production of somatomedin in response to prolactin (Francis & Hill, 1975) suggest that prolactin binding by liver is a biologically important event, as it is in mammary tissue.

Materials and Methods

Materials

Sheep, ox and rat prolactins were obtained from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, MD, U.S.A. Ox somatotropin (growth hormone) was purchased from Miles Laboratories, Elkhart, IN, U.S.A., and [arginine]vasopressin from Spectrum Medical Industries, Los Angeles, CA, U.S.A. Pig corticotropin (ACTH), glucose 6-phosphate, dithiothreitol, neuraminidase and phospholipase C were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Lactoperoxidase, bovine serum albumin (fraction V), normal rabbit serum (freeze-dried), deoxyribonuclease, ribonuclease and Pronase were purchased from Calbiochem, La Jolla, CA, U.S.A. Rabbit anti-(sheep prolactin), 5'-AMP and human γ -globulin (fraction II) were obtained from Schwarz-Mann, Orangeburg, NY, U.S.A. Poly(ethylene glycol) was purchased from J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A., 30% (v/v) H_2O_2 from Mallinckrodt, St. Louis, MO, U.S.A., and highly polymerized calf thymus DNA from Worthington Biochemicals, Freehold, NJ, U.S.A. Acetaldehyde was purchased from Eastman Kodak, Rochester, NY, U.S.A., Na^{125}I (carrier-free) from New England Nuclear, Boston, MA, U.S.A., and *p*-chloromercuribenzoic acid from Kent Laboratories, Vancouver, B.C., Canada. Sephadex G-100 was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals and reagents were purchased from Fisher Scientific Co., Springfield, NJ, U.S.A.

Female Wistar rats were obtained from the animal unit of this University.

Preparation of membrane-enriched fractions from rat liver

Mature female Wistar rats (200–300 g) were used as a source of the tissue preparations. The fractions were prepared in 1 mM- NaHCO_3 , pH 7.5, by a modification of the method of Neville (1960), as described by Emmelot *et al.* (1974). The procedure involved homogenization of liver at 0–4°C in NaHCO_3 (4 ml/g of tissue) in a Potter–Elvehjem homogenizer, and centrifugation at 1500g for 10 min in a Sorvall RC-2B refrigerated centrifuge. The pellet was resuspended in the NaHCO_3 , and centrifuged at 100g for 5 min, followed by 1000g for 10 min, which produced a two-layered pellet. The upper layer of the pellet was

suspended in NaHCO_3 , leaving intact the lower layer, which was much larger in amount, red in colour, and consisted mainly of nuclei and debris. The above centrifugation procedure was repeated twice, yielding eventually a homogeneous pellet, creamy-white in colour. This pellet, suspended in NaHCO_3 , was used as source of membranes. A sample of the membrane suspension was solubilized by incubation in 1M-NaOH at 60°C for 60 min for protein determination by the method of Lowry *et al.* (1951) with bovine serum albumin (fraction V) as standard. The remainder was stored at –20°C at a protein concentration of 3 mg/ml. Samples were thawed and the particles dispersed by use of an all-glass hand-powered homogenizer immediately before use.

Assays for characterizing subcellular fractions

Activity of 5'-nucleotidase (EC 3.1.3.5) was assayed as described by Widnell (1974). Glucose 6-phosphatase (EC 3.1.3.9) was measured by the method of Aronson & Touster (1974), with 2 mM-NaF added to inhibit acid phosphatase (Hübscher & West, 1965). Succinate dehydrogenase (EC 1.3.99.1) was assayed as described by Veeger *et al.* (1969). DNA was determined by the method of Burton (1956).

Preparation of ^{125}I -labelled sheep prolactin

Radioiodinated sheep prolactin was prepared by a lactoperoxidase method described by Rogal & Chrambach (1975). The material was purified by fractionation of the reaction mixture on a column (1.5 cm $\times$ 90 cm) of Sephadex G-100 equilibrated with a buffer [25 mM-Tris/HCl (pH 7.5)/0.1% (w/v) bovine serum albumin] at 4°C. Fractions (1 ml) were collected and assayed for radioactivity, and for activity in the radioimmunoassay and radioreceptor assay. ^{125}I -labelled sheep prolactin was stored at –20°C. Radioactivity was measured in a Nuclear-Chicago gamma counter model 1185.

Procedure for measuring prolactin binding (radio-receptor assay)

The ability of ^{125}I -labelled sheep prolactin to bind to the membrane preparations was measured by the procedure of Shiu *et al.* (1973). Labelled hormone (80000 c.p.m.; 40–60 $\mu\text{Ci}/\mu\text{g}$) was incubated with a sample of the membrane preparation (containing 150 μg of protein) in a final volume of 0.5 ml of buffer (25 mM-Tris/HCl, pH 7.5, containing 10 mM- CaCl_2 or 150 mM-NaCl, and 0.1% bovine serum albumin). For each determination, a parallel incubation was performed in the presence of 5 μg of unlabelled prolactin. The tubes were incubated at 24°C for 6 h, unless otherwise noted. Incubations were terminated by cooling to 0°C in ice. Bound and free ^{125}I -labelled prolactin were separated by centrifugation for 30 min at 3000g at 4°C. Radioactivity in the pellet repre-

sented bound prolactin. Specific binding of prolactin was determined by subtracting the radioactivity (c.p.m.) bound in the presence of excess unlabelled prolactin (non-specific binding) from the total amount bound in the absence of excess hormone. Binding is expressed as a percentage of the total radioactivity added to the incubation mixture.

Procedure for radioimmunoassay

Immunoreactivity of the iodinated prolactin was evaluated by measuring the binding of eluted radioactivity by hormone-specific antibody. Samples (0.1 ml) of eluted fractions were added to an incubation mixture (0.5 ml final volume) containing 0.01 M-sodium phosphate, pH 7.6, 0.15 M-NaCl, 0.1 % NaN_3 , 1 % bovine serum albumin and 1 : 1000 dilution of rabbit anti-(sheep prolactin) serum. After incubation at 24°C for 24 h, the tubes were cooled in ice, and 0.5 ml of cold 0.1 % (w/v) human γ -globulin (fraction II) was added, followed by 1 ml of cold 25 % (w/v) poly(ethylene glycol). The suspensions were mixed thoroughly, and centrifuged at 3000g, 4°C, for 60 min. Radioactivity of the pellets was determined.

Results

Preparation of ^{125}I -labelled prolactin

Four separate peaks of radioactive material were eluted during fractionation of the iodination mixture on Sephadex G-100. One corresponded to iodinated lactoperoxidase, and one to iodide. The other peaks contained material active in the immunoassay and radioreceptor assay. One peak, small in amount, was eluted in the void volume of the column, and may represent aggregated prolactin. The material in the third peak was used in further experiments. Approximate specific radioactivities, calculated by the method of Rogal & Chrambach (1975), of this material were 40–60 $\mu\text{Ci}/\mu\text{g}$. The radioactive hormone could be stored at –20°C for periods of up to 2–3 weeks.

Characterization of membrane preparations

Results in Table 1 show that a plasma-membrane-enriched fraction was obtained from rat liver by the method described. The specific activity of 5'-nucleotidase, a marker enzyme for plasma membranes, was 12-fold higher in the final membrane fraction than in the original homogenate. Marker enzymes for endoplasmic reticulum (glucose 6-phosphatase) and mitochondria (succinate dehydrogenase) showed decreased specific activities. There was some DNA in the final preparation, but the amount was so small, and the ratio of DNA to protein so decreased, that only a slight contamination of membranes with nuclei is indicated. The membrane fraction was also enriched in prolactin binding. This binding function

Table 1. *Distribution of enzyme activity, prolactin binding and DNA in fractions from liver homogenate*

Homogenates of rat liver were prepared and fractionated by centrifugation as described in the Materials and Methods section. The fractions were assayed for several enzyme activities (nmol/min), specific binding of prolactin (fmol) and for DNA content. Results are expressed as means $\pm$ S.E.M.; $n = 4$.

Assay fraction	Specific activity (units/mg of protein)	Yield (%)
5'-Nucleotidase		
Homogenate	73.4 $\pm$ 11	100
1500g pellet	153.0 $\pm$ 16	62.7 $\pm$ 9.3
Membranes	888 $\pm$ 131	11.5 $\pm$ 2.1
Glucose 6-phosphatase		
Homogenate	134.0 $\pm$ 16	100
1500g pellet	132.0 $\pm$ 22	49.6 $\pm$ 10
Membranes	31.2 $\pm$ 9	0.6 $\pm$ 0.2
Succinate dehydrogenase		
Homogenate	0.68 $\pm$ 0.3	100
1500g pellet	0.02 $\pm$ 0.02	9.3 $\pm$ 2.0
Membranes	0	0
Prolactin binding		
Homogenate	65.2 $\pm$ 8	100
1500g pellet	100.3 $\pm$ 16	43.0 $\pm$ 7
Membranes	352.1 $\pm$ 40	4.9 $\pm$ 0.8
DNA content		
Homogenate		100
1500g pellet		85.9 $\pm$ 5.0
Membranes		1.8 $\pm$ 0.4

paralleled the activity of 5'-nucleotidase in all cell fractions.

Specificity of binding of ^{125}I -labelled sheep prolactin to rat liver membranes

The binding of ^{125}I -labelled sheep prolactin to the membrane preparation was inhibited by including increasing amounts of unlabelled sheep, ox or rat prolactin in the assay mixture. Non-lactogenic hormones, such as pig corticotropin, [arginine]vasopressin and ox somatotropin, were without effect (Fig. 1).

Effect of labelled hormone and membrane concentrations on hormone-binding

Specific binding of ^{125}I -labelled sheep prolactin to the liver membrane fraction increased linearly with increasing amounts of membrane protein added (Fig. 2). Non-specific binding increased only slightly in the range of protein concentration used. When increasing amounts of ^{125}I -labelled sheep prolactin were incubated with a fixed amount of membrane

Fig. 1. Specificity of binding of ^{125}I -labelled sheep prolactin to rat liver membranes

Radioactive hormone and samples of membrane preparations were incubated with increasing amounts of the hormones indicated: ●, sheep prolactin; Δ, ox prolactin; ○, rat prolactin; □, ox somatotropin; ■, pig corticotropin and [arginine]vasopressin. Each point is the average of two determinations.

Fig. 2. Effect of membrane-protein concentration on binding of ^{125}I -labelled sheep prolactin

Determination of binding and of protein concentration are described in the Materials and Methods section. ●, Specific binding; ○, non-specific binding.

proteins, both specific and non-specific binding of the hormone increased (Fig. 3). From a Scatchard plot (Scatchard, 1949) of the data (Fig. 4), the apparent K_a (association constant) is $6.9 \times 10^9 \text{ M}^{-1}$. The dissociation constant would be $1.45 \times 10^{-10} \text{ M}$, or roughly 3 ng/ml.

Activation of binding by cations

In the absence of added cations, approx. 2–3% of the ^{125}I -labelled sheep prolactin was specifically bound to the liver membrane preparation. Specific

Fig. 3. Effect of concentration of ^{125}I -labelled sheep prolactin on binding of the hormone

Procedures are described in the Materials and Methods section. ●, Specific binding; ○, non-specific binding.

Fig. 4. Scatchard plot of prolactin binding to liver membranes. Specific binding in Fig. 3 was used for Scatchard analysis. The molecular weight of prolactin used in the calculations was 20000.

Fig. 5. Effects of different ions on binding of ^{125}I -labelled sheep prolactin

Binding of prolactin was measured as described in the Materials and Methods section, except that the type and amount of salt in the incubation mixture were varied. In (a), CaCl_2 (●) or MgCl_2 (○) was added at concentration shown. In (b) binding was determined in the presence of NaCl (▲) or of KCl (△). Non-specific binding is shown in each by □. Each point is the average of three separate determinations.

Fig. 6. Effect of time and temperature on binding of ^{125}I -labelled sheep prolactin to rat liver membranes

Procedures for determining binding are described in the Materials and Methods section. In these experiments, large incubation mixtures (10 times regular volume) were prepared at the different temperatures by mixing the assay ingredients and membranes, pre-equilibrated at specific temperatures, at zero time. Samples were removed from the appropriate flasks to determine specific and non-specific binding at the times and temperatures indicated: 37°C (●); 30°C (○); 23°C (▲); 0°C (△).

binding of the hormone was increased markedly by the addition of NaCl , KCl , CaCl_2 or MgCl_2 , whereas non-specific binding was independent of the addition of salts. The results are shown in Fig. 5. The unusual shape of the curve depicting specific binding in the presence of increasing amounts of CaCl_2 has been observed in every repetition of this experiment, and is unlikely to be an artifact, although there is no

ready explanation for the result. The most effective ions in increasing hormone binding were Mg^{2+} and Ca^{2+} , with half-maximal binding occurring at a concentration of 2.5–4 mM. Maximal binding was quantitatively similar with increasing amounts of NaCl or KCl , but higher concentrations of these salts were required to reach maximum interaction. In the presence of 150 mM- NaCl , the physiological concentration of this salt, 24% of the ^{125}I -labelled prolactin was specifically bound (Fig. 5b). The concentration of CaCl_2 required to produce the same amount of binding was 10 mM, much above physiological values.

Effects of time and temperature of incubation on binding and dissociation of prolactin

The amount of ^{125}I -labelled sheep prolactin bound to the rat liver membrane preparation increased with time and temperature of incubation in the presence of 150 mM- NaCl or 10 mM- CaCl_2 (Fig. 6). At each temperature, the rate of binding decreased after 1–1.5 h of incubation. This change in rate was also independent of the nature of the salts added, as was total binding observed at each temperature.

Dissociation of bound prolactin was also investigated. After 8 h incubation with labelled hormone under regular assay conditions, the membranes were sedimented by centrifugation, then resuspended in fresh medium containing unlabelled prolactin. Dissociation of hormone was determined by re-centrifuging and measuring sedimented and released radioactivity. No dissociation of hormone was observed at any temperature if salts were not included in the incubation mixture. At 0 and 23°C , in the presence of 150 mM- NaCl or 10 mM- CaCl_2 , 5–10% of

the bound prolactin was dissociated in 10–20h incubation. The extent of dissociation was somewhat greater at 37°C, but differed with the salt added. In the presence of 10mM-CaCl₂, 15% of bound prolactin was released during 20h, compared with 30–40% in the presence of 150mM-NaCl. Increasing the amount of unlabelled prolactin in the dissociation incubation mixtures from 3 to 40 µg/ml, or increasing the extent of dilution from 1:4 to 1:100, did not alter the rate or extent of dissociation.

Effect of pH on the binding of prolactin

Specific binding of ¹²⁵I-labelled sheep prolactin to the liver membranes in the presence of 10mM-CaCl₂ or 150mM-NaCl occurred over a relatively wide range of pH. Binding was maximal at pH 6.5, and declined to half-maximal values at pH 5.5 and 8.7 (Fig. 7). At pH 6 or less, labelled hormone was precipitated from solution under the conditions of incubation, thus causing the apparently high non-specific binding.

It was considered possible that the pH optimum observed for ¹²⁵I-labelled prolactin binding might

Fig. 7. Effect of pH on binding of ¹²⁵I-labelled sheep prolactin to rat liver membranes

Determination of binding is described in the Materials and Methods section. Buffers used to obtain the observed pH values were acetate, Tris and NaHCO₃. The pH indicated was determined in the incubation media. ●, Specific binding; ○, non-specific binding.

differ from that for native prolactin. Iodination of tyrosine residues increases the hydrophobicity of the phenoxy ring and decreases the pK of the hydroxy groups in the monoiodinated product. Thus, if the hydroxy groups are involved in the interaction between prolactin and the receptor, iodoprolactin and native prolactin might function differently in the binding assay. To investigate this, iodoprolactin was prepared by the method used for producing ¹²⁵I-labelled prolactin, but with non-radioactive NaI, and the competition for ¹²⁵I-labelled prolactin binding by iodoprolactin and native prolactin was compared. The results (Fig. 8) show that the two forms were equally effective at pH 7.5, with half-maximal occupation of receptors at 21 ng/ml. Native prolactin was more effective at pH 6.5, with half-saturation at 12 ng/ml. Thus native prolactin has a higher affinity for receptor sites at pH 6.5 than does iodoprolactin.

Effects of enzyme, chemical and heat treatment of membranes on ¹²⁵I-labelled sheep prolactin binding

Results in Table 2 show that treatment of the

Fig. 8. Effect of pH on competition of native prolactin and iodoprolactin for binding of ¹²⁵I-labelled sheep prolactin to rat liver membranes

Binding of ¹²⁵I-labelled sheep prolactin was measured (a) at pH 6.5 and (b) at pH 7.5, in the presence of increasing amounts of native sheep prolactin (○) or iodoprolactin (●). Results are expressed as percentage displacement of ¹²⁵I-labelled prolactin specifically bound in the absence of a competitor. Bars represent s.d. for four determinations.

Table 2. *Effect of enzyme treatment on ability of liver membranes to bind ^{125}I -labelled sheep prolactin*

Portions of the membrane suspension were incubated at 37°C for 30 min in the buffer used for binding assays in the presence of different enzymes. For neuraminidase treatment, membranes were suspended in 0.1 M-Tris/acetate, pH 5.6, and incubated at 23°C for 30 min. Concentration of enzymes was 20 µg/ml. After incubation with enzymes, the membranes were sedimented by centrifugation for 20 min at 3000g, 4°C, washed three times with buffer, and resuspended in assay buffer. Specific binding by treated membranes was determined as described in the Materials and Methods section. Binding is expressed as a percentage of specific binding by membranes incubated as described above, but without enzymes added.

Enzyme	Specific binding of ^{125}I -labelled prolactin (% of control)
None	100
Deoxyribonuclease	108
Ribonuclease	98
Pronase	18
Phospholipase C	31
Neuraminidase	132

membrane preparation with enzymes that disrupt protein or phospholipid structure caused a marked decrease in specific binding of labelled hormone. Incubation with neuraminidase, which removes sialic acid residues, resulted in a small increase in prolactin binding. The absence of any effect of ribonuclease or deoxyribonuclease treatment of the membranes suggested that nucleic acids, if present in the preparations, are not involved in hormone binding. A large loss of prolactin binding of membranes exposed to 100°C for only 30 s suggests the requirement for integrity of protein structure for binding (Table 3). Inhibition of binding by low concentrations of dithiothreitol or *p*-chloromercuribenzoic acid (Table 3) indicates the presence of reactive thiol groups (essential for prolactin binding) in the membrane. As shown in Table 3, pretreatment of ^{125}I -labelled prolactin with the thiol-group reagent *p*-chloromercuribenzoic acid had no effect on subsequent binding of the hormone by its receptors.

Discussion

The present study demonstrates that a membrane-enriched fraction from rat liver contains prolactin-accepting sites. This binding shows many of the features used to define hormone-target-cell interaction, as outlined in several reviews (Posner, 1975; Catt & Dufau, 1976, 1977; Ryan & Lee, 1976; Bradshaw & Frazier, 1977). These include structural specificity, high affinity, saturability and some degree of reversibility, particularly at higher temperatures. As described for other hormones (see the reviews), the rate and extent of binding increased if the concentration of either labelled hormone or receptors was increased. Prolactin binding was also dependent on temperature, and exhibited an optimum pH. A specific requirement of this system is the presence of functional thiol groups in the membrane. The form of thiol groups of prolactin seems unrelated to binding. Possible involvement of the tyrosine residues, through their hydroxy groups or their hydrophobic character, in prolactin binding is also suggested by comparison of the competitive effects of native prolactin and iodoprolactin at pH 6.5 and 7.5. There is an essential requirement for ions in prolactin binding by liver membranes. Maximal binding was achieved in the presence of 10 mM- CaCl_2 or 150 mM- NaCl , and at optimal concentrations of each the total amounts of prolactin bound were identical. The mechanism through which cations influence hormone binding is not known. It apparently does not involve ionic strength or osmolarity, since 10 mM- CaCl_2 and 150 mM- NaCl , which activate binding to the same extent, have ionic strengths of 0.03 and 0.15 respectively, and osmolarities of 40 mosm and 300 mosm. It is unlikely that the effects of cations on binding are

Table 3. *Effect of chemical or heat treatment of components of the assay mixture on binding of ^{125}I -labelled sheep prolactin*

Specific binding of labelled prolactin to rat liver membranes was determined in the presence of dithiothreitol or *p*-chloromercuribenzoic acid, and expressed as a percentage of specific binding in the absence of these additions. A portion of the membrane preparation was heated at 100°C for 30 s, and cooled to room temperature before assay of specific binding. In addition, a portion of ^{125}I -labelled prolactin was incubated with *p*-chloromercuribenzoic acid (1 mM) for 30 min at 23°C, then placed on a column (1 cm × 40 cm) of Sephadex G-100, and eluted with 25 mM-Tris/HCl (pH 7.5)/0.1% bovine albumin. Abilities of membranes to bind this prolactin were compared with binding of untreated prolactin.

Treatment of component of assay	Concn. (mM)	Specific binding (% of control)
Heated membranes (100°C, 0.5 min)	—	0
Dithiothreitol added	1.0	34.1
	0.1	57.3
	0.01	94.3
<i>p</i> -Chloromercuribenzoic acid added	1.0	1.0
	0.5	8.0
	0.1	98.4
<i>p</i> -Chloromercuribenzoic acid-treated ^{125}I - labelled prolactin	1.0	100

mediated via an effect of the metal ions on the phase characteristics of the membrane lipids, since univalent cations are without effect on this characteristic (Jacobson & Papahadjopoulos, 1975). The biological significance of prolactin binding by the rat liver membranes is not known. The finding that half-saturation occurred at a prolactin concentration of 3 ng/ml, whereas normal serum prolactin concentrations in female rats are 10–20 ng/ml, might raise questions about the relevance of the binding. This range of saturation is similar to that reported by other workers (2–14 ng/ml), and the discrepancy between membrane saturation and serum concentration has been observed for other hormones (Posner, 1975; Ryan & Lee, 1976; Catt & Dufau, 1976, 1977). Prolactin binding to rat hepatocytes has been demonstrated *in vivo* (Rajaniemi *et al.*, 1974) and *in vitro* (Ranke *et al.*, 1976), and hormones that do not bind to liver membranes *in vitro* are accumulated by liver macrophages *in vivo* (Rajaniemi & Vanha-Perttula, 1972, 1973). Thus prolactin binding would seem to be an early event in a prolactin-induced change in liver function. The effects of prolactin treatment on RNA synthesis, ornithine decarboxylase activity and somatomedin production in rat liver (Chen *et al.*, 1972; Francis & Hill, 1975; Richards, 1975; Thomson & Richards, 1978) suggest that the interaction of prolactin with liver membranes has an important biological function.

Certain differences have been observed in the characteristics of prolactin binding to mammary cell membranes and liver membrane preparations. Optimal binding occurred at pH 6.5 with liver, as opposed to the 7.8 reported for mammary tissue (Shiu & Friesen, 1974a). Treatment of liver membranes with neuraminidase led to an apparent increase in prolactin binding, but the enzyme was without effect on mammary tissue. The effects of salts on prolactin binding also differed in the two tissues. With liver membranes, 10 mM-CaCl₂ and 150 mM-NaCl were equally effective in producing maximal binding. In mammary tissue, univalent cations were not as effective as Mg²⁺ or Ca²⁺ in supporting hormone binding. The significance of these differences cannot be defined. It is possible that identical prolactin receptors exist in mammary-gland and liver cell membranes, but the membranes may provide different environments for the receptors, and thus cause variation in binding requirements. Present evidence cannot differentiate between the above situation and the possibility that the actual receptors may differ in different tissues, although the immunological similarity of prolactin receptors in mammary gland and in ovary has been demonstrated (Bohnet *et al.*, 1978). The species differences observed between animal prolactins in hormone-receptor interaction with rabbit liver membranes supports the suggestion that

a precise relationship of hormone and receptor structure is required for optimal binding.

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