Evidence that non-covalent forces, thiol and disulphide groups affect the structure and binding properties of the prolactin receptor on hepatocytes from pregnant rats

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Incubation of hepatocytes from pregnant rats with dithiothreitol decreased specific ^{125}I -prolactin (^{125}I -prl) binding to such cells by about 20% relative to control. This was not due to a non-specific effect of dithiothreitol on the cell membrane, since reduction also altered the binding of prl to solubilized partially purified receptor. Exposure of hepatocytes to *N*-ethylmaleimide (6 mM) for periods as brief as 1 min decreased the subsequent specific binding of ^{125}I -prl by more than 50%. *N*-Ethylmaleimide was less effective as an inhibitor of binding when applied after hepatocytes had been exposed to ^{125}I -prl, binding being decreased by about 15%. Scatchard analysis demonstrated that the effect of *N*-ethylmaleimide resulted from loss of receptor-binding capacity without any substantial effect on the affinity of the prl receptor for hormone. Dithiothreitol diminished the affinity of lactogenic sites for prolactin without altering cellular binding capacity. These observations suggest that thiol and disulphide groups are present in the prl receptor and that these functional moieties regulate the formation and properties of prl receptor complexes. The species to which ^{125}I -prl had bound were identified by affinity labelling. ^{125}I -prl was covalently coupled into saturable complexes of M_r 65 000 and 50 000. ^{125}I -human growth hormone (^{125}I -hGH) was covalently incorporated into complexes of M_r 300 000, 220 000, 130 000, 65 000 and 50 000. Bovine growth hormone (bGH), but not prl, competed for ^{125}I -hGH uptake into the 300 000-, 220 000- and 130 000- M_r complexes, indicating that these species were somatogenic. Prl, but not bGH, inhibited ^{125}I -hGH uptake into 65 000- and 50 000- M_r complexes. This demonstrated that ^{125}I -hGH in the presence of bGH could affinity-label lactogenic receptors. ^{125}I -prl aggregates in Triton X-100, whereas ^{125}I -hGH does not. Therefore lactogenic complexes to which ^{125}I -hGH was bound in the presence of excess bGH were solubilized in Triton X-100 and characterized sequentially by gel filtration and affinity labelling. Prl receptors were eluted from columns of Sepharose 6B as a species of M_r 380 000. Fractionation of the 380 000- M_r species on sodium dodecyl sulphate polyacrylamide gels resulted in the isolation of complexes of M_r 65 000 and 50 000. Thus non-covalent forces stabilize aggregates of the monomeric prolactin receptor.

Abbreviations used: HBSS, Hanks balanced salt solution; hGH, human growth hormone; bGH, bovine growth hormone; prl, ovine prolactin; SDS, sodium dodecyl sulphate; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid).

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In recent years it has become possible to couple radioactively labelled hormones covalently to membrane receptors and then to fractionate and characterize the complexes thus formed on polyacrylamide gels (Linsley *et al.*, 1981). Such affinity labelling has identified the prl receptor as a species of M_r 35 000–40 000 that does not contain subunits held together by interchain disulphide bonds (Borst & Sayare, 1982; Haepfle *et al.*, 1983;

Yamada & Donner, 1984). In contrast with measurements obtained from affinity-labelling experiments, gel filtration suggests that the prl receptor is a species of M_r 150 000–330 000 (Shiu & Friesen, 1974; Bonifacino *et al.*, 1978; Carr & Jaffe, 1981; Jaffe, 1982; Sasaki *et al.*, 1982). One goal of the present study was to determine the basis for these vastly different descriptions of the receptor for prolactin.

Once formed, prl-receptor complexes become stabilized and hormone is released to the medium very slowly (Powell & Hollander, 1978; Van Der Gugten *et al.*, 1980). Persistent retention of a bound hormone is not unique to the prl-receptor complex. The freely reversible phase of insulin (Donner & Corin, 1980; Corin & Donner, 1982), glucagon (Corin *et al.*, 1982), growth hormone (Donner *et al.*, 1978a, 1980; Donner, 1980) and catecholamine (Williams & Lefkowitz, 1980) binding is followed by the accumulation of complexes from which bound ligand dissociates slowly. A structural basis for this behaviour has not been presented.

In a number of systems, thiol and disulphide groups play a role in regulating the uptake of hormone into receptor complexes. Thiol- and disulphide-group-modifying reagents may, for example, inactivate (Vauquelin *et al.*, 1979) or affect the conversion between affinity states (Moore & Raftery, 1979) of receptors. It is not known if thiol or disulphide groups exist in the prl receptor, and, if so, how they effect the hormone-receptor interaction. A second goal of the present study was to determine if intrachain disulphide bonds or thiol groups regulate the formation and properties of prl-receptor complexes.

Prl receptors are present in unusually large numbers on hepatocytes from pregnant rats (Kelly *et al.*, 1974; Herington *et al.*, 1976; Sasaki *et al.*, 1982). Such cells are therefore a valuable model system in which to characterize structural properties of the prl receptor and have thus been used in the present study.

Experimental procedures

Materials

Pregnant Sprague-Dawley rats were purchased from Charles River Breeding Laboratories and fed Purina laboratory chow *ad libitum*. The sources of all other materials were described previously (Yamada & Donner, 1984).

Methods

Purified hGH was prepared by Dr. Brij Saxena (Cornell University Medical College) (Saxena & Henneman, 1966). hGH monomer, isolated by gel filtration, was used in all studies. This hormone possessed an activity of 1.0 i.u./mg as determined

by weight-gain assay in the hypophysectomized rat (Donner *et al.*, 1978b). Monomeric hGH, bGH (from Dr. Martin Sonenberg, Sloan-Kettering Institute) and ovine prl (38.9 i.u./mg; Sigma) were labelled with ^{125}I by the method of Greenwood *et al.* (1963) and Lesniak *et al.* (1973). Each labelled hormone (sp. radioactivity 75–125 Ci/g) was greater than 95% precipitable in 5% (w/v) trichloroacetic acid. Hepatocytes were isolated from 18–20-day-pregnant rats by modification of the method of Berry & Friend (1969).

The binding of ^{125}I -prl to hepatocytes was assayed by centrifugation (Donner *et al.*, 1978b). Data obtained with ^{125}I -prl alone are designated 'total binding'. ^{125}I -prl binding not inhibited by a high (1 μM) concentration of native prl is termed 'non-specific'. Subtraction of non-specific from the total uptake yields specific binding. In binding experiments, each point is the mean of triplicate determinations. The Bonferroni multiple *t* test (Dunn & Clark, 1974) was used to compare experimental groups in order to determine if the effects of alkylating and reducing agents on lactogenic receptors were significant. Binding and cross-linking experiments were replicated three times, and the results agreed with those presented.

Cross-linking protocols. Hepatocytes (10⁶ cells/ml) were incubated with ^{125}I -labelled hormone (5 nM) in HBSS/1% bovine serum albumin at 23°C for 60 min. The incubation mixture was centrifuged (1500g, 3 min) and the supernatant was aspirated from the resultant cell pellet. The cell pellet was resuspended in ice-cold HBSS and re-centrifuged. This process was repeated twice to remove albumin and unbound hormone from the hepatocytes. The hepatocytes were suspended in HBSS at 15°C, and freshly prepared disuccinimidyl suberate in dimethyl sulphoxide was added to a final concentration of 1 mM. After 15 min the reaction was terminated by addition of 10 vol. of ice-cold 10 mM-Tris/1 mM-EDTA/40 mM-N-ethylmaleimide, pH 7.4. The mixture was centrifuged and suspended in ice-cold 0.25 M-sucrose/40 mM-N-ethylmaleimide. The hepatocytes were disrupted in a Dounce homogenizer with a tight-fitting pestle (30 strokes) and drawn through a 22-gauge needle (10 times) until optical microscopy verified that virtually none of the cells remained intact. The homogenate was centrifuged at 800g for 10 min, and the supernatant was centrifuged at 80 000g for 15 min to yield a crude membrane pellet to which ^{125}I -labelled hormone was covalently bound. This pellet was boiled for 5 min in solubilization medium containing 3% (w/v) SDS, 10% (v/v) glycerol, 6 M-urea, 0.001% Bromophenol Blue, 60 mM-Tris/HCl and 40 mM-N-ethylmaleimide, either with or without dithiothreitol (100 mM). Insoluble components were separated from the

solubilized membrane by centrifugation at 80 000 *g* for 15 min at room temperature.

Electrophoresis and autoradiography. Samples were analysed on 1.5 mm-thick linear-gradient gels containing 4–20% (w/v) acrylamide (acrylamide/bisacrylamide, 100:1, w/w) at 25 mA constant current for 4–5 h with the discontinuous buffer system described by Laemmli (1970). Gels were stained for 1 h in 0.5% Coomassie Blue R dissolved in 25% (v/v) propan-2-ol/7% (v/v) acetic acid. The gel slabs were dried before autoradiography. The M_r standards were run in parallel with experimental samples and their M_r values were: myosin, 200 000; β -galactosidase, 116 250; phosphorylase *b*, 92 500; bovine serum albumin, 66 200; ovalbumin, 45 000; carbonic anhydrase, 31 000; and soya-bean trypsin inhibitor, 21 000. The positions of these standards are shown to the left of each autoradiograph. The apparent M_r values for cross-linked complexes are indicated to the right of each autoradiograph. Autoradiography was performed in cassettes at -80°C with Kodak X-Omat XAR-2 film using du Pont Cronex Lightning Plus enhancing screens. Lead plates [3.2 mm ($\frac{1}{8}$ in) thick] were inserted between cassettes to prevent fogging of films.

Results

In order to determine whether disulphide bonds within lactogenic receptors regulate hormone binding, hepatocytes were incubated with dithiothreitol (2 mM) washed, and then exposed to various concentrations of ^{125}I -prl (Fig. 1*b*). Alternatively, hepatocytes were exposed to ^{125}I -prl for 1 h, dithiothreitol being present during the final 15 min of incubation. Exposure of cells to dithiothreitol before the addition of ^{125}I -prl or co-incubation of ^{125}I -prl and dithiothreitol with hepatocytes diminished specific hormone binding by about 20% relative to control.

The effects of alkylation of thiol groups in lactogenic receptors on hormone binding was investigated. Hepatocytes were incubated with 6 mM *N*-ethylmaleimide, washed and then exposed to ^{125}I -prl (Fig. 1*a*). Alternatively, hepatocytes were exposed to ^{125}I -prl for 1 h, *N*-ethylmaleimide being present during the final 15 min of incubation. Pretreatment with *N*-ethylmaleimide decreased the specific binding of ^{125}I -prl by more than 50% between 0.4 and 8 nM applied prl. The sensitivity of binding to *N*-ethylmaleimide was demonstrated by the ability of this agent to diminish uptake after pretreatment times as brief as 1 min. *N*-Ethylmaleimide was less effective as an inhibitor of binding when applied after hepatocytes had been exposed to ^{125}I -prl. In such instances binding was diminished by about 15%.

Fig. 1. Effects of (a) *N*-ethylmaleimide and (b) dithiothreitol on ^{125}I -prl binding

(a) A hepatocyte preparation was divided into three parts. One part was incubated with various amounts of ^{125}I -prl for 1 h at 23°C (○). A second part was exposed to 6 mM *N*-ethylmaleimide for 2 min, centrifuged (50 *g*, 3 min) and then incubated for 1 h at 23°C with various amounts of ^{125}I -prl (□). A third part was incubated with various amounts of ^{125}I -prl for 1 h at 23°C . During the last 15 min of incubation, 6 mM *N*-ethylmaleimide was present (●). Specific hormone binding in each cell fraction was assayed and each point is the mean of triplicate determinations. (b) A similar experiment was performed in which 6 mM *N*-ethylmaleimide was replaced by 2 mM dithiothreitol. Analysis of variance of data points obtained at the same free-hormone concentrations demonstrated (a) that the effects of *N*-ethylmaleimide were significant after preincubation of cells with this agent or co-incubation of ^{125}I -prl and *N*-ethylmaleimide with hepatocytes ($*P < 0.01$), and (b) that the effects of dithiothreitol on ^{125}I -prl binding were significant ($*P < 0.01$; $**P < 0.05$).

The diminished binding observed after treatment of hepatocytes with dithiothreitol or *N*-ethylmaleimide could have resulted from a loss of binding capacity or a decrease of receptor affinity for prl. In order to distinguish between these possibilities, hepatocytes were exposed to reducing or alkylating agents before incubation with various concentrations of ^{125}I -prl (Fig. 2). *N*-Ethylmaleimide diminished specific hormone binding at both low and high hormone concentrations. In contrast with this, dithiothreitol diminished prl uptake only at low hormone concentrations; binding was not diminished at much higher concentrations of applied prl. Although saturation of the lactogenic receptor may not have been complete under the conditions of assay, Scatchard analysis (inset to Fig. 2) of binding isotherms suggested that *N*-ethylmaleimide diminished receptor number without significantly altering the affinity of lactogenic sites for ^{125}I -prl. Dithiothreitol appeared to diminish the affinity of binding sites for prl, but did not alter receptor number.

The experiments summarized above do not unequivocally demonstrate that the actions of alkylating and reducing agents resulted from modification of lactogenic receptors rather than ^{125}I -prl. In order to demonstrate that *N*-ethylmaleimide and dithiothreitol could alter hormone binding by acting directly on the lactogenic receptor, cells were exposed to a 50 mM concentra-

tion of either reagent for 15 min at 23°C. The cell suspensions were then diluted with 5 ml of HBSS, centrifuged (1500g, 2 min) and resuspended in fresh HBSS/1% albumin. This wash procedure was conducted one or three times, after which hepatocytes were incubated with ^{125}I -prl for 1 h at 23°C. Exposure of cells to *N*-ethylmaleimide reduced ^{125}I -prl binding to 54 and 62% of control after one and three washes respectively. Dithiothreitol diminished ^{125}I -prl binding to 63 and 84% of control levels after one and three washes respectively. In order to evaluate the efficacy of the wash procedure, hepatocytes were incubated with 50 mM-dithiothreitol and then washed once or three times, exactly as described above. The amount of dithiothreitol remaining after each wash was determined on the basis of the ability of this agent to reduce added DTNB to 2-nitro-5-thiobenzoic acid, which was assayed by measurement of A_{412} . The concentration of dithiothreitol was decreased to 5 mM and less than 0.01 mM after one and three washes respectively.

The molecular species to which ^{125}I -prl had bound in the experiments described above were identified by affinity labelling. Disuccinimidyl suberate covalently coupled ^{125}I -prl into complexes of M_r 65000 and 50000, as well as into a number of other lower- M_r complexes (Fig. 3, lane *d*). The ability of a large excess of native prl to inhibit the formation of the 65000- and 50000- M_r

Fig. 2. Effects of pre-exposure of hepatocytes to *N*-ethylmaleimide or dithiothreitol on prl binding. Hepatocytes were incubated with 50 mM-*N*-ethylmaleimide for 2 min ($\square$), 50 mM-dithiothreitol for 15 min ($\bullet$), or without additions for 15 min ($\circ$). The incubation mixtures were centrifuged (50g, 2 min) and the supernatant was aspirated from the resultant cell pellets. The cells were incubated with various concentrations of ^{125}I -prl for 80 min at 23°C. Specific hormone binding was assayed and each point is the mean of triplicate determinations. Data are plotted as bound against free prl (main Figure) or as described by Scatchard (1949) (inset).

Fig. 3. Cross-linking ^{125}I -labelled hormones to hepatocytes Hepatocytes ($10^6/\text{ml}$) were incubated for 1 h at 23°C with: lane (a), ^{125}I -hGH (5 nM) + bGH ($1\ \mu\text{M}$); lane (b), ^{125}I -hGH (5 nM); lane (c), ^{125}I -hGH (5 nM) + prl ($1\ \mu\text{M}$). In a separate experiment, hepatocytes were incubated with: lane (d), ^{125}I -prl (5 nM); lane (e), ^{125}I -prl + prl ($1\ \mu\text{M}$). After cross-linking, a crude membrane fraction was isolated from each incubation mixture. Equal amounts of radioactivity from solubilized membranes were fractionated on linear 4–20%-(w/v)-polyacrylamide gradient gels.

complexes (lane e) identified these as resulting from interaction binding of ^{125}I -prl with components of the prl receptor.

^{125}I -hGH binds to both growth hormone and prl receptors and was covalently incorporated into complexes of M_r 300 000, 220 000, 130 000, 65 000 and 50 000 on hepatocytes from pregnant rats (Fig. 3, lane b). bGH inhibited the incorporation of ^{125}I -hGH into the 300 000-, 220 000- and 130 000- M_r complexes without affecting the isolation of the 65 000- or 50 000- M_r species (lane a). ^{125}I -hGH was isolated in 300 000-, 220 000- and 130 000- M_r complexes, but not in 65 000- or 50 000- M_r complexes in the presence of excess prl (lane c). A lower- M_r (40 000) species was present in incubation mixtures containing ^{125}I -hGH alone (lane b) or in the presence of bGH (lane a), prl (lane c) or hGH (not shown), and was therefore non-specific.

^{125}I -hGH was incubated with hepatocytes in the presence of bGH to affinity-label prl receptors (as in Fig. 3, lane a). Lactogenic receptors containing bound and covalently bound ^{125}I -hGH were solubilized into Triton X-100 and fractionated on a column of Sepharose 6B (Fig. 4). ^{125}I -hGH with bGH instead of ^{125}I -prl was used to identify

Fig. 4. Fractionation of prl receptors on Sepharose 6B Hepatocytes were incubated with 5 nM- ^{125}I -hGH and bGH ($1\ \mu\text{M}$) for 60 min at 23°C . After cross-linking, a crude membrane fraction isolated from each incubation mixture was shaken in 50 mM-Tris (pH 7.4)/1% Triton X-100 for 60 min at 4°C and centrifuged (100 000g, 20 min). The supernatant was applied to a column (1 cm $\times$ 100 cm) of Sepharose 6B equilibrated with 50 mM-Tris/HCl buffer (pH 7.9)/0.1% Triton X-100. Fractions (0.8 ml) from the column were assayed for ^{125}I (b), precipitated in 5% (w/v) trichloroacetic acid (4°C , 10 min) and centrifuged (800g, 20 min). The pellets produced were neutralized with 0.1 M-NaOH, solubilized in the extraction medium employed for gel electrophoresis and fractionated on a linear 4–20%-(w/v)-polyacrylamide gradient gel. The part of autoradiograph identifying components of the prl receptor is shown (a). Fractions 58, 60, 63, 66, 70, 73–75 and 82 from the Sepharose column were fractionated on lanes a, b, c, d, e, f and g of the gel respectively.

lactogenic receptors in this experiment, since the latter hormone aggregates in Triton X-100. After isolation of a small amount of uncharacterized material in the void volume of the Sepharose column, two peaks of radioactive label were recovered. The first peak was eluted with a K_{av} of 0.48 (M_r 380 000), and the second peak was eluted within the same volume as authentic ^{125}I -hGH.

In order to determine if either peak of radioactivity recovered from Sepharose fractionation contained ^{125}I -hGH-prl-receptor complexes, radioactivity peaks 1 and 2 were subjected to SDS/polyacrylamide-gel electrophoresis (Fig. 4a). Peak 1 contained lactogenic complexes of M_r 65000 and 50000, non-specifically coupled hormone ($M_r < 40000$) and ^{125}I -hGH that had not been covalently coupled to receptors and therefore ran as free hormone during electrophoresis. Peak 2 contained ^{125}I -hGH. Results identical with those described above were obtained after affinity labelling and gel filtration of lactogenic receptors

Fig. 5. Fractionation of reduced prl receptors on Sepharose 6B

Hepatocytes were incubated with 5 nM - ^{125}I -hGH and bGH ($1\text{ }\mu\text{M}$) for 60 min at 23°C . After cross-linking, a crude membrane fraction isolated from each incubation mixture was shaken in 50 mM -Tris ($\text{pH } 7.4$)/ 1% Triton X-100 for 60 min at 4°C and centrifuged ($100000g$, 20 min). The supernatant from this procedure was incubated with dithiothreitol (50 mM) for 1 h at 4°C , applied to a column of Sepharose 6B and then treated exactly as described in the legend to Fig. 4. Sepharose fractions 60, 63, 66, 68, 72, 74, 76, 78 and 82 correspond to lanes (a), (b), (c), (d), (e), (f), (g), (h) and (i) of the autoradiograph respectively.

from hepatocytes from non-pregnant female rats (results not shown).

We wished to evaluate the role that disulphide bonds play in regulating the properties of solubilized prl-receptor complexes. Lactogenic receptors into which ^{125}I -hGH was bound or covalently cross-linked were solubilized into Triton X-100 and then incubated with dithiothreitol for 1 h at 4°C before fractionation on a column of Sepharose 6B (Fig. 5). After a small forepeak that was eluted in the void of the column, three peaks of radioactivity were recovered and characterized by gel electrophoresis. Peak 1 contained lactogenic complexes of M_r 65000 and 50000, a non-specific complex of M_r about 40000 and ^{125}I -hGH. The second peak contained some 40000- M_r complex and low- M_r species, including ^{125}I -hGH. The third peak contained ^{125}I -hGH. Comparison of Figs. 4 and 5 demonstrates that exposure to dithiothreitol resulted in dissociation of ^{125}I -hGH from lactogenic complexes with the resultant recovery of increased amounts of free ^{125}I -hGH and other low- M_r species, which may have been degradation products of prl-receptor complexes. The elution of the aggregate containing ^{125}I -hGH covalently bound to monomeric components of the hormone receptor was unchanged by exposure to dithiothreitol.

Discussion

Exposure of liver cells to dithiothreitol diminished the affinity of lactogenic binding sites for prl. This effect was manifested without loss of cellular binding capacity for this hormone, demonstrating that the prl receptor was not inactivated by such treatment. It is unlikely that the decrease of receptor affinity resulted from non-specific effects of dithiothreitol on the hepatocyte plasma membrane, since reduction of solubilized lactogenic receptors produced a similar effect (Fig. 5). These observations suggest that intrachain disulphide bonds stabilize prl-receptor complexes.

N-Ethylmaleimide alkylates reactive thiol groups in hormone receptors (Massague *et al.*, 1980). Exposure of hepatocytes to *N*-ethylmaleimide inhibited the subsequent, specific binding of ^{125}I -prl by lactogenic receptors. Analysis of the results of binding experiments by the graphical method suggested by Klotz (1982) (present Fig. 2) or with Scatchard (1949) plots (present Fig. 2) demonstrated that *N*-ethylmaleimide decreased the apparent number of prl receptors on hepatocytes without having any substantial effect on the affinity of the prolactin receptor for the hormone. A proportionately smaller loss of binding capacity occurred when hepatocytes were treated with *N*-ethylmaleimide after incubation with ^{125}I -prl.

Two explanations may be offered for this. Occupation of binding sites may directly block access of *N*-ethylmaleimide to its site of action and diminish its effectiveness. This implies that a reactive thiol group is present at the site at which the hormone-receptor interaction occurs. Alternatively, binding may induce a conformational change within the receptor, rendering inaccessible the site with which *N*-ethylmaleimide reacts. In either event, our observations provide direct evidence for the presence of thiol groups in the prolactin receptor that are essential for ligand-receptor interaction.

Exhaustive washing after exposure of hepatocytes to either *N*-ethylmaleimide or dithiothreitol failed to prevent these agents from altering subsequent ^{125}I -prl binding. This observation suggests that at least some of the actions of each reagent resulted from a direct action on the receptor for prl. It is possible, however, that, during co-incubation of *N*-ethylmaleimide or dithiothreitol with ^{125}I -prl and hepatocytes, hormone binding was also altered by actions of reducing or alkylating agents on the hormone.

The molecular species to which prolactin bound in the experiments described above were identified. ^{125}I -prl was covalently coupled into complexes of M_r 65000 and 50000, as well as being incorporated into a number of lower- M_r species. The ability of prl to inhibit the uptake of ^{125}I -prl into the 65000- and 50000- M_r complexes demonstrated that these species resulted from binding of hormone to components of the lactogenic receptor. The formation of the lower- M_r complexes was not inhibited by unlabelled hormones (Fig. 3, lanes *a-c*), suggesting that these resulted from non-saturable (non-specific) hormone binding. Subtracting the M_r of prolactin from that of the specific complexes into which it was incorporated suggests that the hormone had bound to species of M_r 43000 and 28000. The former M_r is in good agreement with that of affinity-labelled lactogenic complexes identified on hepatocytes from normal rats (Yamada & Donner, 1984), on mouse (Liscia *et al.*, 1982), rat or rabbit liver membranes (Borst & Sayare, 1982; Haepfle *et al.*, 1983) and on rabbit mammary membranes (Haepfle *et al.*, 1983). The amount of the 50000- M_r complex varied from one hepatocyte preparation to another, and it may therefore be that this species resulted from interaction of hormone with a degradation product derived from the intact receptor, although this remains unproven.

Unfortunately it was not possible to use ^{125}I -prl to characterize the properties of the solubilized prolactin receptor by gel filtration, since this hormone aggregates in Triton detergent. An alternative method for identifying the lactogenic receptor was therefore developed which took

advantage of the ability of hGH to bind to lactogenic and somatogenic receptors (Yamada & Donner, 1984). Thus ^{125}I -hGH was incorporated into complexes of M_r 300000, 220000, 130000, 65000 and 50000 on hepatocytes from pregnant rats (Fig. 3). Complexes were classified as lactogenic or somatogenic on the basis of the ability of bGH or prl, which bind exclusively to GH and prl receptors (Donner, 1983; Yamada & Donner, 1984) respectively to compete with ^{125}I -hGH for incorporation into each species. The 300000-, 220000- and 130000- M_r complexes were somatogenic, since bGH, but not prl, competed for ^{125}I -hGH uptake into these species. The ability of prl, but not bGH, to inhibit ^{125}I -hGH uptake into the 65000- and 50000- M_r complexes identified these as lactogenic. The identity of the specific molecular species observed in affinity-labelling experiments with ^{125}I -prl and ^{125}I -hGH with excess bGH demonstrated that hGH could be used to characterize the properties of the receptor for prolactin.

Lactogenic complexes solubilized into Triton X-100 from the livers of rats in a number of physiological states, including pregnancy, were eluted from gel-filtration columns as species of 150000–380000 M_r (Shiu & Friesen, 1974; Bonafacino *et al.*, 1978; Carr & Jaffe, 1981; Jaffe, 1982; Sasaki *et al.*, 1982; Fig. 4). Affinity labelling demonstrated that a lactogenic receptor of M_r 380000 contained complexes of M_r 65000 and 50000. This directly demonstrates for the first time that the solubilized receptor for prl is composed of monomeric components held together by non-covalent interactions. This observation is consistent with the known properties of Triton X-100, which is a mild detergent that does not inhibit protein-protein aggregation and can therefore be used profitably to determine whether non-covalent interactions stabilize receptor structure (Pilch & Czech, 1980). Prolactin receptors solubilized into Chaps{3-[(3-cholamidopropyl)dimethylammonio]propanesulphonic acid}, a detergent that does not favour protein aggregation, have an M_r of 37000 (Liscia *et al.*, 1982). It is not known whether aggregation of prl receptors has physiological significance. This would not be surprising, however, since hormone receptors aggregate in clathrin-coated regions of the cell membrane during their down-regulation (Pastan & Willingham, 1981). Also, receptor aggregation mediates the actions of a number of peptide-hormone receptors, including those for insulin (Kahn *et al.*, 1978) and epidermal growth factor (Schecter *et al.*, 1979).

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