Structures of the somatotropin receptor and prolactin receptor on rat hepatocytes characterized by affinity labelling

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Human somatotropin competed for ^{125}I -human somatotropin binding to hepatocytes from female or male rats. Bovine somatotropin and prolactin each inhibited part, but not all, of the uptake of ^{125}I -human somatotropin. The binding of ^{125}I -prolactin was inhibited by human somatotropin and prolactin, but not by bovine somatotropin. Bovine somatotropin and human somatotropin, but not prolactin, competed for ^{125}I -bovine somatotropin binding sites. ^{125}I -labelled hormones were covalently coupled to membrane receptors with higher efficiency on hepatocytes from female than from male rats, allowing structural descriptions of lactogenic and somatogenic binding sites that had not been possible previously. Disuccinimidyl suberate covalently coupled ^{125}I -human somatotropin into saturable complexes of M_r 300 000, 220 000, 130 000, 65 000 and 50 000. Bovine somatotropin inhibited the incorporation of ^{125}I -human somatotropin into complexes of M_r 300 000, 220 000 and 130 000, whereas low concentrations of prolactin competed for incorporation into the 65 000- and 50 000- M_r species. ^{125}I -bovine somatotropin was incorporated into complexes of M_r 300 000, 220 000 and 130 000. Human somatotropin and bovine somatotropin, but not prolactin, inhibited the production of these complexes. ^{125}I -prolactin binding produced complexes of M_r 65 000 and 50 000. Native prolactin and human somatotropin, but not bovine somatotropin, inhibited uptake of ^{125}I -prolactin into these species. Thus direct affinity labelling, as well as competition for covalent coupling, suggests that the 300 000-, 220 000- and 130 000- M_r species are components of the somatotropin receptor and that the 65 000- and 50 000- M_r complexes result from hormone binding to the prolactin receptor. By subtracting the M_r of prolactin, it was calculated that the hormone was bound to species of M_r 43 000 and 28 000. These M_r values were not affected by reduction of solubilized membranes, suggesting that the structure of the prolactin receptor is not stabilized by interchain disulphide bonds between subunits. Subtracting the M_r of somatotropin from somatogenic complexes indicated that the hormone had bound to species of M_r 280 000, 200 000 and 100 000. The 300 000- and 220 000- M_r complexes were not isolated from reduced membranes, whereas the amount of the 130 000- M_r species was augmented. These observations could suggest that a major component of the somatotropin receptor is a trimeric aggregate in which some subunits are retained in a larger complex by interchain disulphide bonds.

^{125}I -labelled peptide hormones can be covalently bound to receptors in the membranes of target cells by using organic cross-linking reagents (Linsley *et al.*, 1981). Such affinity labelling can

Abbreviations used: HBSS, Hanks balanced salt solution; SDS, sodium dodecyl sulphate.

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describe receptor structure and therefore supplements binding assays, which measure site affinity or number. Affinity labelling has been used in studies of receptors for insulin (Yip *et al.*, 1978; Jacobs *et al.*, 1979, 1980; Pilch & Czech, 1979, 1980), insulin-like growth factors I and II (Massague & Czech, 1982), human chorionic gonadotropin (Rebois *et al.*, 1981), epidermal growth fac-

tor (Linsley *et al.*, 1979), glucagon (Johnson *et al.*, 1981) and angiotensin II (Paglin & Jamieson, 1982).

^{125}I -labelled human somatotropin, which interacts with somatogenic and lactogenic sites (Kelly *et al.*, 1974; Ranke *et al.*, 1976), has been covalently coupled to receptors on hepatocytes from male rats (Donner, 1983). Electrophoresis, on non-gradient SDS/polyacrylamide gels, of radioactivity solubilized from such cells in the absence of reductant, revealed complexes of M_r 300 000, 220 000 and 130 000. A number of less-well-defined lower- M_r species were isolated from membranes solubilized in the presence of reductant. In these experiments the efficiency of cross-linking was low and it was therefore not possible to completely distinguish lactogenic from somatogenic complexes. In particular, the properties of the faint 220 000- M_r species remained unresolved.

The goals of this study were to affinity-label somatotropin and prolactin receptors on hepatocytes isolated from female rats with ^{125}I -bovine somatotropin and ^{125}I -prolactin, in addition to ^{125}I -human somatotropin. We also wished to increase the efficiency of hormone cross-linking to these receptors and then, by using SDS/polyacrylamide gradient gels, to resolve somatogenic from lactogenic complexes and particularly to determine if the 220 000- M_r complex isolated previously (Donner, 1983) was a component of the somatotropin or the prolactin receptor.

Experimental procedures

Materials

The sources of all materials were described previously (Donner, 1983).

General methods

Purified human somatotropin was prepared by Dr. Brij Saxena (Cornell University Medical College) (Saxena & Henneman, 1966). Human somatotropin monomer was isolated by gel filtration and 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 human somatotropin, bovine somatotropin (from Dr. Martin Sonenberg, Sloan-Kettering Institute), and ovine prolactin (38.9 i.u./mg; Sigma) were labelled with ^{125}I by the method of Greenwood *et al.* (1963) and Lesniak *et al.* (1973). The specific radioactivities of these species (65–125 Ci/g) were determined by assaying the incorporation of ^{125}I into a 20 μg sample of hormone by precipitation in trichloroacetic acid or by gel filtration. ^{125}I -labelled hormone was separated from free ^{125}I by elution of the reaction mixture from a column (0.9 cm $\times$ 27 cm) of

Sephadex G-75. Each labelled hormone was more than 95% precipitable in 5% (w/v) trichloroacetic acid. Hepatocytes were prepared by modification of the method of Berry & Friend (1969) as previously described (Donner *et al.*, 1978a). The binding of labelled hormones to hepatocytes was assayed by centrifugation (Donner *et al.*, 1978b).

Cross-linking protocols

Hepatocytes ($\sim 10^6$ cells/ml) were incubated with ^{125}I -labelled hormone (5 nM unless stated otherwise) in HBSS (Grand Island Biological Co.)/1% bovine serum albumin at 23°C for 60 min. The incubation mixture was centrifuged (1500g for 3 min) and the supernatant was aspirated from above the resultant cell pellet. The cell pellet was resuspended in ice-cold HBSS without albumin and re-centrifuged. This process was repeated two additional times to remove albumin and unbound hormone from the hepatocytes. The hepatocytes were resuspended 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 (1500g, 3 min) and resuspended 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/0.06 M-Tris/HCl/40 mM-*N*-ethylmaleimide with or without 100 mM-dithiothreitol.

Electrophoresis and autoradiography

The samples were analysed on gradient gels, containing 4–20% acrylamide (100:1 acrylamide/bisacrylamide ratio), that were 1.5 mm thick; the discontinuous buffer system described by Laemmli (1970) was used unless otherwise stated. Electrophoresis was carried out at a constant current of 25 mA for 4–5 h. The gels were stained for 1 h in 0.5% Coomassie Blue R dissolved in isopropyl alcohol/acetic acid/water (25:7:68, by vol.). The gel slabs were dried before autoradiography. M_r standards were run in one lane to enable estimation of the M_r of cross-linked species. The standards and their M_r values were: fibronectin (440 000), myosin (200 000), β -galactosidase

(116250), phosphorylase *b* (92500), bovine serum albumin (66200), and ovalbumin (45000). The positions and M_r values of these standards are shown to the left of each autoradiograph. The apparent M_r values of cross-linked complexes are indicated to the right of each autoradiograph. Generally, between 5000 and 40000 c.p.m. of radioactive label was applied to each lane of a gel. Autoradiography was carried out in cassettes with Kodak X-O-mat XAR-2 film using a Dupont Cronex Lightning Plus enhancing screen. Lead plates [0.317 cm ($\frac{1}{8}$ in) thick] were inserted between cassettes to prevent fogging of the films. Films were exposed from 4 to 21 days at -80°C before development.

Results

^{125}I -prolactin or ^{125}I -bovine somatotropin were incubated with hepatocytes from male rats in the presence of various amounts of unlabelled human somatotropin, bovine somatotropin or prolactin to determine whether these labelled hormones distin-

guish somatogenic and lactogenic receptors. Prolactin or human somatotropin inhibited ^{125}I -prolactin binding to receptors on hepatocytes (Fig. 1a). Non-specific binding of prolactin to hepatocytes from male animals was invariably a substantial fraction of the total radioactive uptake. In contrast with human somatotropin, bovine somatotropin did not compete for lactogenic binding sites. Human somatotropin and bovine somatotropin, but not prolactin, inhibited the uptake of ^{125}I -bovine somatotropin in a dose-dependent manner (Fig. 1b). There was a greater amount of ^{125}I -bovine somatotropin (12.7 pmol/ 10^9 cells) than prolactin (5.7 pmol/ 10^9 cells) specifically bound to

Fig. 1. Somatogenic and lactogenic receptors on hepatocytes from male rats

Hepatocytes ($\sim 10^6$ cells/ml) were incubated with 5 nM- ^{125}I -prolactin (a) or ^{125}I -bovine somatotropin (b) in the presence of various concentrations of human somatotropin (O), prolactin (●) or bovine somatotropin (◇). After 1 h at 23°C , binding of labelled hormone was assayed by centrifugation. Each point represents the mean result of triplicate determinations.

Fig. 2. Competition for somatogenic and lactogenic receptors on hepatocytes from female rats

Hepatocytes ($\sim 10^6$ /ml) were incubated with 5 nM- ^{125}I -human somatotropin (a), ^{125}I -prolactin (b) or ^{125}I -bovine somatotropin (c) in the presence of various concentrations of human somatotropin (O), prolactin (●), or bovine somatotropin (◇). After 1 h at 23°C , binding of labelled hormone was assayed by centrifugation. Each point represents the mean result of triplicate determinations.

hepatocytes after incubation of cell suspensions with a 5 nM concentration of either hormone for 1 h at 23°C.

Experiments similar to those illustrated in Fig. 1 were conducted with hepatocytes from female rats. Human somatotropin inhibited ^{125}I -human somatotropin binding to hepatocytes in a concentration-dependent manner (Fig. 2a). Bovine somatotropin or prolactin competed for ^{125}I -human somatotropin binding, prolactin being more effective in this regard. The binding of ^{125}I -prolactin was inhibited by human somatotropin or prolactin, which were equally effective competitors (Fig. 2b). Bovine somatotropin competed little, if at all, for prolactin uptake. Bovine somatotropin and human somatotropin, but not prolactin, competed for ^{125}I -bovine somatotropin binding sites. After incubation with 5 nM- ^{125}I -human somatotropin, ^{125}I -prolactin or ^{125}I -bovine somatotropin, 81, 45 or 12.6 pmol of labelled hormone respectively were specifically bound per 10^9 cells.

^{125}I -human somatotropin was covalently bound to hepatocytes from female rats. The M_r values for the complexes into which ^{125}I -human somatotropin was bound (300 000, 220 000, 130 000, 65 000

and 50 000) were similar in female (Fig. 3, lanes a and i) and male (Donner, 1983) rats. Incubations were also conducted with ^{125}I -human somatotropin and various concentrations of bovine somatotropin, prolactin or human somatotropin to distinguish somatogenic from lactogenic complexes. Bovine somatotropin inhibited the incorporation of ^{125}I -human somatotropin into complexes of M_r 300 000, 220 000 and 130 000 in a dose-dependent manner, but had little or no effect on uptake into complexes of M_r 65 000 or 50 000 (Fig. 3, lanes e–g). Low concentrations of prolactin eliminated ^{125}I -human somatotropin uptake into complexes of M_r 65 000 and 50 000 (Fig. 3, lanes b–d). Much larger concentrations of prolactin may have inhibited production of higher- M_r species. Human somatotropin prevented ^{125}I -human somatotropin from generating lower- or higher- M_r complexes (Fig. 3, lane h).

The cross-linking of ^{125}I -bovine somatotropin and ^{125}I -prolactin to sites on hepatocytes from female rats was compared with that of ^{125}I -human somatotropin. ^{125}I -human somatotropin was incorporated into the five predominant complexes (Fig. 4, lane d). Prolactin inhibited the formation of complexes of M_r 65 000 and 50 000 (lane c). ^{125}I -

Fig. 3. ^{125}I -human somatotropin cross-linking to hepatocytes from female rats

Hepatocytes ($\sim 10^6/\text{ml}$) were incubated with ^{125}I -human somatotropin (5 nM) for 1 h at 23°C with: lane a, no unlabelled hormone (control); b, 9 nM-prolactin; c, 900 nM-prolactin; d, 6000 nM-prolactin; e, 9 nM-bovine somatotropin; f, 90 nM-bovine somatotropin; g, 900 nM-bovine somatotropin; h, 900 nM-human somatotropin; i, no unlabelled hormone (control). Cross-linking of bound hormone was then carried out and a crude membrane fraction was prepared from each incubation mixture. Membranes were solubilized in the absence of reductant and equal volumes of extract (not equal amounts of radioactivity) from each incubate were subjected to electrophoresis on a linear 4–20% polyacrylamide gradient gel.

Fig. 4. Cross-linking ^{125}I -human somatotropin, ^{125}I -bovine somatotropin and ^{125}I -prolactin to hepatocytes from female rats. Hepatocytes ($\sim 10^6/\text{ml}$) were incubated for 1 h at 23°C with: lane a, ^{125}I -bovine somatotropin (5 nM) and bovine somatotropin ($1.7\text{ }\mu\text{M}$); b, ^{125}I -bovine somatotropin (5 nM); c, ^{125}I -human somatotropin (5 nM) and prolactin ($1.7\text{ }\mu\text{M}$); d, ^{125}I -human somatotropin (5 nM); e, ^{125}I -human somatotropin (5 nM) and bovine somatotropin ($1.7\text{ }\mu\text{M}$); f, ^{125}I -prolactin (5 nM); g, ^{125}I -prolactin (5 nM) and prolactin ($1.7\text{ }\mu\text{M}$). Cross-linking was carried out and a crude membrane fraction was isolated from each incubation mixture. Membranes were solubilized in the absence of reductant, and equal amounts of radioactive label from each incubation mixture were subjected to electrophoresis on a linear 4–20% polyacrylamide gradient gel.

bovine somatotropin was incorporated into the species of M_r 300 000, 220 000 and 130 000 and only in well-developed films were complexes of M_r 65 000 or 50 000 faintly visible (lane b). The uptake of ^{125}I -bovine somatotropin to yield the higher- M_r species was saturable with respect to hormone concentration and thus was inhibited by bovine somatotropin (lane a). Cross-linking of bound ^{125}I -human somatotropin in the presence of excess bovine somatotropin (lane e) or ^{125}I -prolactin (lane f) produced complexes of M_r 65 000 and 50 000 and little of the higher- M_r species. The relative proportions of these complexes varied somewhat from one membrane preparation to another (results not shown). Prolactin inhibited the incorporation of ^{125}I -prolactin into the 65 000- and 50 000- M_r species (lane g).

Non-specific cross-linking was best evaluated on gels comparing equal amounts of radioactive

label (Fig. 4) rather than equal volumes of solubilized membrane protein (Fig. 3) from various incubation mixtures. This was true, since solubilized membranes from incubation mixtures containing somatotropin (Fig. 3, lane h) contained much less radioactivity than similar incubation mixtures with ^{125}I -human somatotropin alone or in the presence of prolactin or bovine somatotropin. A low- M_r (40 000) complex resulted from non-specific cross-linking between hormone and another species, since native bovine somatotropin (Fig. 4, lane a), prolactin (Fig. 4, lane g) or human somatotropin (results not shown) did not prevent incorporation of labelled hormone into this species.

The specificity of the uptake of ^{125}I -prolactin into complexes of M_r 65 000 and 50 000 was tested in competition experiments (Fig. 5). Native human somatotropin (lane b) or prolactin (lane c), but not bovine somatotropin (lane d), inhibited the

Fig. 5. ^{125}I -prolactin cross-linking to hepatocytes from male and female rats

Hepatocytes ($\sim 10^6/\text{ml}$) from a female rat were incubated with ^{125}I -prolactin for 1 h at 23°C . Equal volumes of reduced or unreduced solubilized extract from incubation mixtures (containing: lane a, ^{125}I -prolactin, reduced; b, ^{125}I -prolactin and $1.7\ \mu\text{M}$ -human somatotropin, reduced; c, ^{125}I -prolactin and $1.7\ \mu\text{M}$ -prolactin, reduced; d, ^{125}I -prolactin and $1.7\ \mu\text{M}$ -bovine somatotropin, reduced; e, ^{125}I -prolactin, unreduced) were characterized on a linear 4–20% (w/v)-polyacrylamide gradient gel. Equal amounts of radioactive label ($3600 \pm 100\ \text{c.p.m.}$) from incubation mixtures with hepatocytes (from: lane f, a female rat, reduced; g, a male rat, reduced) were characterized on a linear 5–20%-polyacrylamide gradient gel after being reduced with dithiothreitol (50 mM).

generation of the 65000- and 50000- M_r species under either reducing or non-reducing conditions (lanes a and e). Although reduction did not significantly affect the mobility of lactogenic complexes, it was invariably observed that the intensity of the 65000- M_r species was diminished by solubilization of membranes in the presence of dithiothreitol. An interesting difference between the cross-linking of ^{125}I -prolactin to hepatocytes from female (lane f) and male (lane g) rats was also observed. Receptor binding demonstrated the presence of lactogenic sites on hepatocytes from rats of either sex (Figs. 1 and 2). Despite this, it was not possible to clearly observe the incorporation of ^{125}I -prolactin into specific complexes by affinity labelling in experiments with hepatocytes from male animals.

The properties of (^{125}I -bovine somatotropin)-receptor complexes were further characterized

(Fig. 6). In the presence of reductant, cross-linked complexes of M_r 300000 and 220000 were not observed; only the 130000- M_r species was isolated after covalently coupling ^{125}I -bovine somatotropin to hepatocytes from male (lane e) or female (lane d) rats. Bovine somatotropin and human somatotropin, but not prolactin, inhibited the incorporation of ^{125}I -bovine somatotropin into the 130000- M_r species. Finally, the intensity of the 130000- M_r complex was greater after cross-linking ^{125}I -bovine somatotropin to hepatocytes from female than from male rats. Observations similar to these were made in experiments with at least four hepatocyte preparations from rats of either sex and employing two different samples of ^{125}I -bovine somatotropin or ^{125}I -human somatotropin. The difference in intensity of the 130000- M_r complex on hepatocytes from male or female animals was therefore significant.

Fig. 6. Cross-linking of ^{125}I -bovine somatotropin to hepatocytes from male and female rats. Hepatocytes ($\sim 10^6$ cells/ml) from female (lanes a–d) or male (lanes e–h) rats were incubated with ^{125}I -bovine somatotropin (5 nM) for 1 h at 23°C in the presence of: lane a, $1.7\text{ }\mu\text{M}$ -prolactin; b, $1.7\text{ }\mu\text{M}$ -bovine somatotropin; c, $1.7\text{ }\mu\text{M}$ -human somatotropin; d, ^{125}I -bovine somatotropin alone; e, ^{125}I -bovine somatotropin alone, control; f, $1.7\text{ }\mu\text{M}$ -human somatotropin; g, $1.7\text{ }\mu\text{M}$ -bovine somatotropin; h, $1.7\text{ }\mu\text{M}$ -prolactin. Cross-linking was carried out and a crude membrane fraction was isolated from each incubation mixture. Membranes were solubilized in the presence of dithiothreitol, and equal amounts of radioactive label from each extract were subjected to electrophoresis on a linear 5–15% (w/v)-polyacrylamide gel (acrylamide/bisacrylamide ratio 37.5:1).

Discussion

Previously (Donner, 1983) ^{125}I -human somatotropin was covalently coupled to sites on hepatocytes from male rats. In that study it was not possible to unambiguously resolve somatogenic from lactogenic receptors and, in particular, to determine whether a $220000\text{-}M_r$ complex resulted from hormone binding to the somatotropin receptor or the prolactin receptor. Significantly, these difficulties have been overcome by use of hepatocytes from female rats. Prolactin and somatotropin receptors were labelled more efficiently on cells from female than male rats (Figs. 5 and 6). One explanation for this could be that the greater number of prolactin receptors on hepatocytes from female rats facilitated covalent labelling. We consider this unlikely, since somatotropin receptors, present in about equal numbers on hepatocytes from rats of either sex, were also labelled more efficiently on cells from female animals. Since somato-

genic and lactogenic receptors on cells from female or male rats appear identical, the accessibility of these sites to the cross-linking reagent is probably different. The high efficiency of cross-linking to cells from female animals reduced the fraction of non-specifically coupled radioactivity and assisted the interpretation of experimental observations.

Competition for binding characterized the sites to which human somatotropin, bovine somatotropin and prolactin bind on hepatocytes from male or female rats (Kelly *et al.*, 1974; Donner, 1983; Figs. 1 and 2). Bovine somatotropin or prolactin inhibited a component of the uptake of ^{125}I -human somatotropin. Human somatotropin and prolactin, but not bovine somatotropin, competed for ^{125}I -prolactin binding to hepatocytes from female or male rats. Prolactin, however, did not compete for ^{125}I -bovine somatotropin uptake, whereas bovine and human somatotropin were equally effective in this regard. These observations and the activities of these hormones (Kleinberg &

Frantz, 1971; Li, 1972) suggest that bovine somatotropin and prolactin bind to somatogenic and lactogenic receptors respectively. Human somatotropin, which possesses weak lactogenic (Kleinberg & Frantz, 1971) as well as growth-promoting activity (Evans *et al.*, 1943), binds to somatogenic and prolactin receptors (Kelly *et al.*, 1974; Ranke *et al.*, 1976; Donner, 1983).

The specificities of human somatotropin, bovine somatotropin and prolactin for binding to cells from female rats distinguished somatotropin from prolactin receptors. ^{125}I -human somatotropin was taken into complexes of 300000, 220000, 130000, 65000 and 50000- M_r . Bovine somatotropin inhibited the incorporation of ^{125}I -human somatotropin into complexes of M_r 300000, 220000 and 130000 without affecting the isolation of the 65000- and 50000- M_r species. Low concentrations of prolactin competed for ^{125}I -human somatotropin incorporation into complexes of M_r 65000 and 50000 (Fig. 3). These experiments suggest that (^{125}I -human somatotropin)-receptor complexes can be segregated into somatogenic (M_r 300000, 220000 and 130000) and lactogenic (M_r 65000 and 50000) subgroups.

The assignment of the complexes into which ^{125}I -human somatotropin was incorporated as somatogenic or lactogenic was verified by affinity labelling with ^{125}I -bovine somatotropin or ^{125}I -prolactin. ^{125}I -bovine somatotropin bound to receptors to yield species of M_r 300000, 220000 and 130000 (Fig. 4). Complexes with such apparent M_r values were identified when ^{125}I -human somatotropin was covalently bound to hepatocytes in the presence of sufficient prolactin to saturate lactogenic sites. Bovine somatotropin competed for the binding of ^{125}I -bovine somatotropin (Figs. 1 and 2) and prevented the incorporation of the labelled hormone into the 300000, 220000 and 130000- M_r species. On the other hand, ^{125}I -prolactin, or ^{125}I -human somatotropin in the presence of sufficient bovine somatotropin to saturate somatogenic receptors, was incorporated into complexes of M_r 65000 and 50000. Prolactin competed for both receptor binding and incorporation of ^{125}I -prolactin into the 65000- and 50000- M_r complexes in a dose-dependent manner. Thus direct affinity labelling as well as competition for incorporation into covalent complexes support the assignment of the 300000-, 220000- and 130000- M_r species as components of the somatotropin receptor and of the 65000- and 50000- M_r complexes as components of the prolactin receptor.

High concentrations of prolactin may have inhibited ^{125}I -human somatotropin uptake into complexes of M_r 300000, 220000 and 130000. On well-developed autoradiographs, components of prolactin preparations were in a complex of M_r

130000 and components in bovine somatotropin preparations had been taken into species of M_r 65000 and 50000. Trace contamination of one pituitary hormone by the other probably accounts for this.

^{125}I -prolactin was incorporated into saturable complexes of M_r 65000 and 50000. The apparent M_r of the receptor was not significantly affected by reduction of solubilized membranes before or during electrophoresis (Fig. 5). From this we infer that interchain disulphide bonds do not fix putative subunits into a macromolecular complex. It is not known whether the 50000- M_r complex resulted from binding to a unique receptor component or from a degradative process. Variability in the proportions of the 65000- and 50000- M_r complexes after affinity labelling could indicate that proteolysis of receptor components occurs in hepatic membrane preparations. By subtracting the M_r of prolactin, it can be calculated that the hormone was covalently bound to species of M_r 43000 and 28000. The former value is in good agreement with the M_r of prolactin receptors from lactating-mouse liver plasma membranes (Liscia & Vonderhaar, 1982; Liscia *et al.*, 1982; M_r = 37000), rat (Borst & Sayare, 1982; M_r = 36000) or rabbit (Haeuple *et al.*, 1983; M_r = 35000) liver and rabbit mammary membranes (Haeuple *et al.*, 1983).

Previously (Donner, 1983) it was considered possible that the 220000- M_r complex formed by binding somatotropin to hepatocytes might result from interaction with components of the receptor for prolactin. This proposal was considered viable, although unproven, since partially purified prolactin receptors have apparent- M_r values of about 220000 (Shiu & Friesen, 1974). It has now been demonstrated that the 220000- M_r complex described here is a component of the somatotropin receptor. Subtracting the M_r of somatotropin from that of the somatogenic complexes suggests that the hormone had bound to species of M_r 280000, 200000 and 100000. The 300000- and 220000- M_r complexes were not isolated from reduced membranes (Fig. 6), whereas the amount of the 130000- M_r species was augmented (Donner, 1983). As with insulin receptors (Kasuga *et al.*, 1982) or somatotropin receptors from male rats (Donner, 1983), monomeric subunits were identified in the absence of reductant in solubilized membranes from cells from female animals (Figs. 3 and 4). These observations imply that the somatotropin receptor is an aggregate in which some subunits are retained in a larger complex by interchain disulphide bonds.

Affinity labelling identified a 130000- M_r somatotropin-receptor complex on IM-9 lymphocytes (Hughes *et al.*, 1983). A somatotropin receptor of M_r 300000 from pregnant-rabbit liver mem-

branes contained a component of M_r 75000 (Waters & Friesen, 1979). A number of complexes of M_r less than 130000 were isolated after affinity-labelling receptors on microsomal fractions of rabbit liver (Haeuple *et al.*, 1983; Hughes *et al.*, 1983), rabbit mammary gland and rat liver (Hughes *et al.*, 1983). In affinity-labelling experiments with hepatocytes from male rats, Donner (1983) identified a number of minor, low- M_r complexes into which somatotropin was bound. In evaluating structure, it is possible that not all of the components of the somatotropin receptor have been identified, and others may be labile. The isolation of major complexes of M_r 300000, 220000 and 130000 might suggest that a major component of the somatotropin receptor is a trimeric aggregate of 100000- M_r subunits. Further study is required to evaluate the minor, low- M_r complexes identified in other studies and to determine if additional subunits of this receptor are present.

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