An investigation of sites that bind human somatotropin (growth hormone) in the liver of the pregnant rabbit

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The binding of ^{125}I -labelled human somatotropin (growth hormone) to a crude membrane preparation from the liver of pregnant rabbit, and to receptors solubilized from this fraction by Triton X-100, was dependent on time, temperature and receptor concentration. At 4°C a steady state was reached after 20 h, and maximum specific binding (as a percentage of total tracer added) was approx. 50% for both membrane-bound and solubilized receptors. Solubilization did not significantly affect the binding properties of the receptor at low concentrations of Triton X-100 (less than 0.05%, v/v, in the assay tube). However, at higher concentrations (approx. 0.1%, v/v), the detergent lowered the ability of some hormones, for example ovine prolactin, to displace ^{125}I -labelled human somatotropin, but did not affect other hormones such as bovine somatotropin. Some somatogenic hormones, such as bovine somatotropin, and some lactogenic hormones, such as ovine prolactin, displaced ^{125}I -labelled human somatotropin from membrane-bound and solubilized receptor preparations. Furthermore, 85% of ^{125}I -labelled bovine somatotropin was displaced from membrane-bound receptors by ovine prolactin, and ^{125}I -labelled ovine prolactin was almost completely displaced by bovine somatotropin. Scatchard analysis of the binding data for human somatotropin suggested a single class of binding sites in the membrane-bound receptor preparation, with an affinity (K_a) of $1.9 \times 10^9 \text{ M}^{-1}$ and a capacity of 1726 fmol/mg of protein; these values were slightly increased by solubilization ($K_a = 3.2 \times 10^9 \text{ M}^{-1}$, capacity = 2103 fmol/mg of protein). Scatchard analysis of binding to membrane-bound receptors also indicated a single class of high-affinity binding sites for bovine somatotropin ($K_a = 4.8 \times 10^9 \text{ M}^{-1}$, capacity = 769 fmol/mg) and for ovine prolactin ($K_a = 6.1 \times 10^9 \text{ M}^{-1}$, capacity = 187 fmol/mg).

It is generally accepted that receptors for polypeptide hormones are associated with the cell membrane, and crude membrane preparations of various tissues from different species have been shown to contain sites that bind ^{125}I -labelled human somatotropin (Posner *et al.*, 1974). The highest specific (displaceable by excess unlabelled hormone) binding of ^{125}I -labelled human somatotropin was found in the liver of the late-pregnant rabbit (Tsushima & Friesen, 1973). This is consistent with several of the known actions of somatotropin, such as the stimulation of somatomedin production (McConaghey & Sledge, 1970) and protein synthesis (Korner, 1959) in the liver, and effects on the activities of certain enzymes in the liver, such as glutamate dehydrogenase (Cheek & Graystone, 1969), ornithine decarboxylase and RNA polymerase (Jänne & Raina, 1969).

A crude microsomal fraction from liver of late-pregnant rabbit has been widely used as the basis

for a radioreceptor assay for human somatotropin. Since human somatotropin has lactogenic as well as somatogenic activity it is important to determine to which type of receptor the hormone is binding. For example, it has been suggested that the high specific binding of human somatotropin in the liver of the female rat is due to lactogenic rather than somatogenic binding sites (Herington *et al.*, 1976; Bonifacino *et al.*, 1981), although other workers have identified equal numbers of both lactogenic and somatogenic sites in this tissue (Ranke *et al.*, 1976). Tsushima & Friesen (1973) originally identified the human somatotropin-binding sites in rabbit liver as somatogenic, but it has since been shown that receptors for lactogenic hormones, such as ovine prolactin, are also present (Posner *et al.*, 1974; Parke & Forsyth, 1975).

There is disagreement as to the exact nature of the human somatotropin-binding sites in the liver of the female rabbit. Waters & Friesen (1979) found

two types of receptor, one specific for somatotropin, with a low affinity for ovine prolactin, and one specific for prolactin only. On the other hand, Tsushima *et al.* (1980) and Herington & Veith (1977) found only minimal binding of ovine prolactin to their receptor preparations. The situation is further complicated by the use of receptor preparations solubilized with the non-ionic detergent Triton X-100, since this has been shown to affect the stability of certain hormones, such as ovine prolactin (Shiu & Friesen, 1974b).

In the present paper we have attempted to clarify this situation, through a detailed characterization of the human somatotropin-binding sites in the liver of late-pregnant rabbit. We have studied the effects of detergent solubilization on the binding properties of the receptors by comparing membrane-bound and solubilized receptors, including preparations containing very low concentrations of detergent. We have also investigated the species specificity of the receptors in some detail, using a wide variety of somatotropins and prolactins, including the homologous hormones rabbit somatotropin and prolactin. Preliminary reports of part of this work have been presented previously (Cadman & Wallis, 1980; Wallis & Cadman, 1981).

Materials and methods

Materials

Human somatotropin (1.6 i.u./mg) was a gift from the National Institute of Biological Standards and Control, Hampstead, London, U.K. Bovine somatotropin was prepared by a chromatographic procedure (Wallis & Dixon, 1966). Ovine somatotropin (NIH-GH-S9), rat somatotropin (NIH-GH-B3), rabbit somatotropin (AFP-684-C), ovine prolactin (NIH-P-S9), bovine prolactin (NIH-P-B3), rat prolactin (NIH-RPRL-B1) and rabbit prolactin (AFP-1974-C) were gifts from Dr. A. E. Wilhelmi, Dr. A. Parlow and the National Institute of Arthritis, Metabolism and Digestive Diseases, NIH, Bethesda, U.S.A. Human placental lactogen (batch 5212) was obtained from the Nutritional Biochemicals Corp., Cleveland, OH, U.S.A. Porcine somatotropin (28B-2350), bovine serum albumin (Cohn fraction V) and merthiolate (thimerosal) were purchased from Sigma (London) Chemical Co., Poole, Dorset, U.K. Sephadex G-50 (Fine grade) and Sephadex G-100 (Superfine grade) were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Na¹²⁵I (carrier-free; IMS 30) was from The Radiochemical Centre, Amersham, Bucks., U.K. Iodogen (1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril) was purchased from Pierce and Warriner (U.K.), Chester, Cheshire, U.K. Poly(ethylene glycol) 6000 and Triton X-100 were obtained from BDH Chemicals, Poole, Dorset, U.K. Rabbit

anti-(human somatotropin) serum (batch no. RD 15/16) was purchased from Wellcome Reagents, Beckenham, Kent, U.K. Pregnant New Zealand White rabbits were from Cork Farm, Chilham, Canterbury, Kent, U.K.

Iodination of hormones

¹²⁵I-labelled human somatotropin, bovine somatotropin and ovine prolactin were prepared by the 'iodogen' method of Fraker & Speck (1978). A solution of iodogen (30 µl; 1 mg/25 ml in dichloromethane) was dried in a thin film in a small polypropylene tube. The hormone (10 µg) was dissolved in 10 µl of sodium phosphate buffer (0.5 M-NaH₂PO₄, adjusted to pH 7.4 with NaOH), and Na¹²⁵I (1 mCi; 10 µl) was added. The reaction was allowed to proceed for 10 min and was then terminated by the addition of 500 µl of sodium phosphate buffer (0.5 M-NaH₂PO₄, adjusted to pH 7.4 with NaOH). The reaction mixture was transferred to a Sephadex G-50 (Fine grade) column (0.78 cm² × 10–14 cm), equilibrated and eluted with sodium phosphate buffer (0.05 M-NaH₂PO₄, adjusted to pH 7.6 with NaOH), containing 0.05% bovine serum albumin and 0.6 mM-merthiolate, to separate labelled hormone from unchanged iodide. Fractions corresponding to the peak of labelled hormone were pooled, and were re-chromatographed before use on a column (0.78 cm² × 95–100 cm) of Sephadex G-100 (Superfine grade), equilibrated and eluted with 0.025 M-Tris/HCl buffer, pH 7.6, containing 0.1% bovine serum albumin and 0.6 mM-merthiolate.

Specific radioactivity of each tracer was measured by self-displacement in a radioimmunoassay, as described by Ketelslegers *et al.* (1975). Fig. 1 shows the result of a typical experiment to estimate the specific radioactivity of ¹²⁵I-labelled human somatotropin; 100 µl (approx. 10 000 c.p.m.) of ¹²⁵I-labelled human somatotropin was added to 100 µl of antibody to human somatotropin (diluted 1:120 000) and increasing concentrations of labelled or unlabelled hormone. Sodium phosphate buffer (0.025 M-NaH₂PO₄, adjusted to pH 7.6), containing 0.05% bovine serum albumin and 0.6 mM-merthiolate, was added to give a final volume of 300 µl and tubes were incubated for 72 h at 4°C. Antibody-bound hormone was precipitated with poly(ethylene glycol) 6000 by adding to each tube a cold (4°C) mixture containing 25% (w/v) poly(ethylene glycol), 0.15% bovine γ-globulin and 0.05 M-Tris/HCl, pH 8.5. After mixing and standing for 1 h at 4°C the tubes were centrifuged (20 min, 1000 g, 4°C). Supernatants were removed by aspiration and precipitates were counted for radioactivity (Nuclear-Chicago γ-Radiation Counter, model 1195; counting efficiency for ¹²⁵I 50–55%).

Parallel curves for the inhibition of binding of ¹²⁵I-labelled human somatotropin were obtained by

Fig. 1. Determination of specific radioactivity of ^{125}I -labelled human somatotropin

^{125}I -labelled human somatotropin (approx. 10000 c.p.m.) was incubated with antibody and increasing concentrations of labelled (●) or unlabelled (○) hormone. Specific binding was determined. Each value represents the mean of quadruplicate determinations.

using both labelled and unlabelled human somatotropin. Half-maximal displacement of ^{125}I -labelled human somatotropin was seen with 0.29 ng of unlabelled and 27×10^3 c.p.m. of labelled hormone. Thus 1 ng of unlabelled hormone is equivalent to 93×10^3 c.p.m., giving a specific radioactivity of 76 Ci/g.

Preparation of membrane-bound receptors

A crude membrane fraction was prepared from the liver of a late-pregnant rabbit by the method of Tsushima & Friesen (1973). The liver was homogenized in 5 vol. of ice-cold 0.3 M-sucrose, using a Polytron PT-10 homogenizer (Kinematica G.m.b.H.) for 1 min at speed setting 7. The homogenate was filtered through eight layers of cheese-cloth and then centrifuged at 1500 g for 20 min at 4°C. The resulting supernatant was then centrifuged at 15000 g for 20 min at 4°C and finally at 100000 g for 90 min at 4°C. The microsomal fraction (100000 g pellet), referred to as membrane-bound receptors, was resuspended in 'assay buffer' (0.025 M-Tris/HCl buffer, pH 7.6, containing 0.1% bovine serum albumin, 0.6 mM-merthiolate and 10 mM- CaCl_2), frozen in 2 ml portions and stored at -20°C.

Solubilization of receptors

Receptors were solubilized by the method of Shiu & Friesen (1974b). In preliminary experiments the microsomal fraction was diluted with 'assay buffer' (see above) to a protein concentration

of 5–10 mg/ml (as estimated by the method of Lowry *et al.*, 1951), and Triton X-100 was added to a final concentration of 1% (v/v). The suspension was stirred for 30 min (at room temperature) and then centrifuged at 200000 g for 2 h at 4°C. The supernatant, referred to as 'solubilized receptors', was frozen in 2 ml portions and stored at -20°C. Approx. 70% of the total protein in the microsomal fraction was solubilized by 1% (v/v) Triton X-100 and the preparation was diluted to give a final detergent concentration of around 0.1% (v/v) in the assay tube. In later experiments it was found that by using an original protein concentration of 10 mg/ml and 0.5% (v/v) Triton X-100, 60% of the total protein could be solubilized. It was then possible to dilute the receptor preparation to give a final detergent concentration of less than 0.05% (v/v) in the assay tube. The 'solubilized receptors' referred to below are those containing the lower concentration of detergent unless otherwise specified.

Binding studies

The incubation procedure was identical for both membrane-bound and solubilized receptors. All dilutions of receptor preparations, standards and labelled hormones were carried out in 'assay buffer' (see above). Each assay tube contained 200 μl of assay buffer, 100 μl of membrane-bound or solubilized receptors (100–200 μg of protein), 100 μl of unlabelled hormone (0.32–1000 ng/tube) and 100 μl of ^{125}I -labelled hormone (approx. 30000 c.p.m./tube). Tubes were mixed and incubated for 20 h at 4°C. The assay using membrane-bound receptors was terminated by the addition of 4 vol. of ice-cold 0.025 M-sodium acetate/acetic acid buffer, pH 5.4, containing 0.1% bovine serum albumin. The assay using solubilized receptors was terminated by the addition of 0.5 ml of ice-cold sodium phosphate buffer (0.1 M- NaH_2PO_4 , adjusted to pH 7.5 with NaOH), followed by 1 ml of ice-cold 25% (w/v) poly(ethylene glycol) 6000. The tubes were mixed thoroughly and allowed to stand at 4°C for 30 min before centrifugation. In both assays free and bound hormone were separated by centrifugation (1000 g for 25 min at 4°C). Supernatants were removed by aspiration and the precipitates were subjected to γ -counting. Binding that could not be displaced by an excess (1 μg) of unlabelled hormone was considered to be non-specific. Specific binding was calculated from total binding minus non-specific binding.

Removal of Triton X-100

Triton X-100 was removed from solubilized receptor preparations by the method of Holloway (1973) using Amberlite XAD-2 beads. Washed Amberlite beads (15 g) were incubated with twice the volume of solubilized receptor extract for 2 h at

4°C. Extract and beads were separated on a sintered glass funnel.

Scatchard analysis of binding data

Scatchard analysis (Scatchard, 1949) was performed on saturation curves, in which a constant amount of receptor was incubated with increasing concentrations of labelled hormone, non-specific binding being determined as described previously. In calculating the results the 'bindability', that is, the maximum bindable fraction of each hormone, was taken into account. This was estimated by incubating the labelled hormone with an excess of receptor preparation and determining the percentage of total radioactivity specifically bound. 'Bindability', using membrane-bound receptors, was approx. 50%, 45% and 35% for human and bovine somatotropin and ovine prolactin respectively. It was not possible to calculate the bindability of bovine somatotropin or ovine prolactin to solubilized receptors, as the high concentrations of detergent caused by increased receptor concentration affected the binding of both these hormones. Binding of human somatotropin was not affected and the bindability was approx. 50%, as for membrane-bound receptors. Saturation curves were analysed, since only labelled hormone is used in this system and correction for bindability is easily made. In analysis of displacement curves, which use both labelled and unlabelled hormone (assumed to have 100% bindability), correction for bindability is more complex.

Results

Effects of time and temperature on hormone binding

Specific binding of ^{125}I -labelled human somatotropin to both membrane-bound and solubilized receptors was shown to be time and temperature dependent (Fig. 2). In all cases non-specific binding (results not shown) was constant after 8 h. Binding to the membrane-bound receptors was only slightly faster at 25°C than at 4°C. A steady state (equilibrium) was reached after 18 h and the mean specific binding achieved at equilibrium was similar at 4°C ($23.2 \pm 0.14\%$ of total radioactivity added; results are means $\pm$ S.E.M. of triplicate determinations) and at 25°C ($23.9 \pm 0.49\%$). Non-specific binding was slightly lower at 4°C ($10.6 \pm 0.35\%$) than at 25°C ($14.0 \pm 0.22\%$). In the case of the solubilized receptors binding was faster, and a steady state was reached in a shorter time at 25°C, although the mean specific binding at equilibrium was lower at 25°C ($27.9 \pm 0.39\%$) than at 4°C ($37.1 \pm 0.35\%$). Non-specific binding ($22.1 \pm 0.75\%$ at 4°C and $25.8 \pm 0.23\%$ at 25°C) was higher than for the membrane-bound receptors, possibly due to poly-

Fig. 2. Effect of time and temperature on the binding of ^{125}I -labelled human somatotropin to (a) membrane-bound and (b) solubilized receptors

^{125}I -labelled human somatotropin (approx. 30000 c.p.m.) was incubated with receptor preparation ($150\mu\text{g}$ of protein) at 4°C (○) or at 25°C (●) and specific binding (percentage of total radioactivity) was determined. Each value represents the mean of triplicate determinations.

(ethylene glycol) 6000 precipitation of aggregated ^{125}I -labelled hormone.

Effect of receptor concentration on binding of hormone

Fig. 3 shows the effect of increasing concentration of membrane-bound and solubilized receptors on the specific binding of ^{125}I -labelled human somatotropin. Binding to both types of receptor preparation increased approximately linearly up to $200\mu\text{g}$ of protein. All subsequent binding studies on ^{125}I -

Fig. 3. Effect of receptor concentration on the binding of ^{125}I -labelled human somatotropin to (a) membrane-bound and (b) solubilized receptors

^{125}I -labelled human somatotropin (approx. 30 000 c.p.m.) was incubated for 20 h at 4°C with increasing concentrations of receptor preparation. Specific binding (percentage of total radioactivity) was calculated. Each value represents the mean of quadruplicate determinations

labelled human somatotropin were therefore carried out using between $100\mu\text{g}$ and $200\mu\text{g}$ of protein per tube. Maximum specific binding was 46% of total counts for membrane-bound and 54% for solubilized receptors. At higher concentrations of receptor (more than $600\mu\text{g}$ of protein/tube) an increase in protein concentration gave increased non-specific binding and therefore did not result in greater specific binding.

Specificity of receptors

Fig. 4 shows the displacement of ^{125}I -labelled somatotropin from membrane-bound and solubilized receptors by somatotropins, prolactins and choriomammotropins (placental lactogens) from a variety of species. The displacement curves for both types of receptor preparation are similar (although not identical), suggesting that solubilization does not significantly affect the binding properties of the receptor.

Bovine (ox) and ovine (sheep) somatotropin both

Fig. 4. Specificity of displacement of ^{125}I -labelled human somatotropin from (a and b) membrane-bound and (c and d) solubilized receptors

^{125}I -labelled human somatotropin (approx. 30 000 c.p.m.) was incubated with receptor preparation ($150\mu\text{g}$ of protein) in the presence of increasing amounts of human somatotropin (●), bovine somatotropin (■), ovine somatotropin (▲), porcine somatotropin (☆), rabbit somatotropin (○), rat somatotropin (◆), bovine prolactin (□), ovine prolactin (△), rabbit prolactin (▼), rat prolactin (◇) and human choriomammotropin (★). Each value represents the mean of quadruplicate determinations.

effected considerable displacement of ^{125}I -labelled human somatotropin. Porcine (pig), rat and rabbit somatotropin also competed for binding sites but with a low potency compared with human somatotropin.

Bovine and ovine prolactin both competed effectively for binding sites for ^{125}I -labelled human somatotropin, although bovine prolactin was more potent than ovine prolactin. The difference between the two prolactins is surprising, since bovine and ovine prolactin are almost identical in sequence (Wallis, 1978) and have very similar potency in

radioimmunoassay. Rat and rabbit prolactin showed no competition, and human chorionmammotropin displaced ^{125}I -labelled human somatotropin with approx. 0.1% of the potency of human somatotropin itself.

The specificity of the binding of ^{125}I -labelled bovine somatotropin and ovine prolactin to membrane-bound receptors was also investigated. Fig. 5 shows the rather unexpected result that ovine prolactin was capable of considerable displacement of ^{125}I -labelled bovine somatotropin, and that bovine somatotropin was almost as effective as ovine prolactin at displacing ^{125}I -labelled ovine prolactin.

Effect of Triton X-100 on receptor specificity

Fig. 6 compares the displacement of ^{125}I -labelled human somatotropin, by various hormones, from solubilized receptor preparations containing different concentrations of Triton X-100. At a final detergent concentration of approx. 0.1% (v/v), ovine prolactin displaced ^{125}I -labelled human somatotropin with only 0.5–1.0% of the potency of human somatotropin itself (Fig. 6a). However, with the receptor preparation containing less than 0.05% (v/v) Triton X-100, ovine prolactin was far more effective in displacing the labelled hormone (Fig. 6b). The binding of bovine and ovine somatotropin did not appear to be significantly affected by the higher concentration of detergent.

Removal of the detergent from the receptor preparation containing approx. 0.1% (v/v) Triton X-100 by Amberlite beads gave displacement curves similar to those obtained using the receptor preparation containing less than 0.05% Triton X-100 (results not shown), although non-specific binding was greatly increased (44%).

Scatchard analysis of binding data

Fig. 7 shows Scatchard analysis of saturation curves (results from at least three experiments, means $\pm$ S.E.M.). The results for membrane-bound receptors indicate a single class of binding site for human somatotropin with a high affinity [$(1.88 \pm 0.03) \times 10^9 \text{ M}^{-1}$] and a high capacity ($1726 \pm 60 \text{ fmol/mg}$). Solubilization caused a slight increase in affinity [$(3.21 \pm 0.27) \times 10^9 \text{ M}^{-1}$] and in capacity ($2103 \pm 172 \text{ fmol/mg}$). As explained previously it was not possible to calculate affinities and capacities for bovine somatotropin and ovine prolactin with solubilized receptors. However, the results for membrane-bound receptors indicate a single class of binding site in each case, with affinities of $(4.75 \pm 0.46) \times 10^9 \text{ M}^{-1}$ and $(6.06 \pm 0.16) \times 10^9 \text{ M}^{-1}$ for bovine somatotropin and ovine prolactin respectively. These high-affinity bovine somatotropin-binding sites were of fairly high capacity ($769 \pm 51 \text{ fmol/mg}$), although those for ovine prolactin were of very much lower capacity ($187.4 \pm 15.2 \text{ fmol/mg}$).

Fig. 5. Specificity of displacement of (a) ^{125}I -labelled bovine somatotropin and (b) ^{125}I -labelled ovine prolactin from membrane-bound receptors

^{125}I -labelled bovine somatotropin (approx. 50 000 c.p.m.) or ^{125}I -labelled ovine prolactin (approx. 90 000 c.p.m.) were incubated with membrane-bound receptors (300 μg of protein) and increasing concentrations of bovine somatotropin (●) or ovine prolactin (○). Each value represents the mean of quadruplicate determinations.

Fig. 6. Effect of (a) approx. 0.1% and (b) <0.05% Triton X-100 on the specificity of displacement of ^{125}I -labelled human somatotropin.

^{125}I -labelled human somatotropin (approx. 30000 c.p.m.) was incubated with solubilized receptor preparation (150 μg of protein), containing the appropriate concentration of detergent, in the presence of increasing amounts of human somatotropin (●), bovine somatotropin (■), ovine somatotropin (▲), rat somatotropin (◆), ovine prolactin (△), rat prolactin (◇) or human chorionmammotropin (★). Each value represents the mean of quadruplicate determinations.

Discussion

High specific binding of ^{125}I -labelled human somatotropin is found in a variety of tissues from different species. In some cases the binding sites have been identified as lactogenic, for example the mammary gland receptors of the rabbit (Shiu & Friesen, 1974a). However, the hepatic sites that bind human somatotropin in rat and rabbit appear to be more complex. In the present paper we have attempted to characterize these receptors in the late-pregnant rabbit, to clarify the rather conflicting reports about receptors from this very rich source.

The microsomal fraction from a liver homogenate was used as this was shown by Tsushima & Friesen (1973) to bind ^{125}I -labelled somatotropin in the same manner as purified plasma membranes. Also this fraction has the highest specific binding of labelled human somatotropin and the highest level of 5'-nucleotidase activity (Posner *et al.*, 1974). We obtained high specific binding of ^{125}I -labelled

somatotropin to membrane-bound and to solubilized receptors. In both cases binding was dependent on the time and temperature of incubation, and on the concentration of the receptor preparation, in general agreement with the results of other workers (Herington & Veith, 1977; Tsushima *et al.*, 1980).

Our results suggest that there are very few specific somatogenic or specific lactogenic sites in the liver, since human somatotropin could be displaced almost completely by somatogenic and by lactogenic hormones. This does not agree with the work of Tsushima & Friesen (1973), who characterized these receptors as somatogenic; however, they only tested human prolactin. Other workers have since shown specific binding of ^{125}I -labelled ovine prolactin to rabbit liver membranes, which suggests that receptors able to bind lactogenic hormones are also present (Posner *et al.*, 1974; Parke & Forsyth, 1975).

Since solubilization with the non-ionic detergent Triton X-100 has been widely used as a first step

Fig. 7. Scatchard analysis of saturation curves for (a) human somatotropin (membrane-bound receptors), (b) human somatotropin (solubilized receptors), (c) bovine somatotropin (membrane-bound receptors) and (d) ovine somatotropin (membrane-bound receptors)

Increasing amounts of ^{125}I -labelled hormone were incubated with the appropriate receptor preparation ($150\mu\text{g}$ of protein) and specific binding was determined. Each value represents the mean of quadruplicate determinations. Insets show Scatchard analysis of the saturation curves. The values calculated for each affinity constant (K_d) and capacity (Cap) represent the means of three separate experiments. Values for capacity are given as fmol/mg of protein and the units for K_d are M^{-1} .

in purification of the receptors we investigated the effect of this on their binding properties. It was found that solubilization did not significantly affect the

binding properties of the receptors, providing the final concentration of detergent in the assay tubes was low (less than 0.05%, v/v). However, at higher

concentrations (approx. 0.1%, v/v) the detergent affected the ability of certain hormones, such as ovine prolactin, to displace labelled human somatotropin. It has been shown (Shiu & Friesen, 1974b) that Triton X-100 at a concentration of approx. 0.1% (v/v) affects the stability of ovine prolactin, but does not affect human somatotropin. This might explain the results of Herington & Veith (1977) and Tsushima *et al.*, (1980), who found that ovine prolactin displaced ^{125}I -labelled human somatotropin from solubilized receptors [containing approx. 0.1% (v/v) Triton X-100] with a very low potency compared with human somatotropin itself. This is in accordance with our own results at the higher concentration of detergent.

Tsushima *et al.* (1980) separated two types of receptor by affinity chromatography, one of which bound human and bovine somatotropin, whereas the other bound human somatotropin only. Herington & Veith (1977) also concluded that two types of receptor were present from Scatchard analysis of their binding data. Waters & Friesen (1979), with a solubilized receptor preparation containing less than 0.05% Triton X-100, also separated two types of receptor, one somatogenic with a low affinity for ovine prolactin, the other lactogenic with a high selective affinity for ovine prolactin. Our work suggests that some discrepancies in the characterization of receptors could be explained by the concentration of detergent in the solubilized receptor preparations used. Also, discrepancies between the results of different groups could be due to variations in the rabbits used. For example, Tsushima & Friesen (1973) and Tsushima *et al.* (1980) used liver of pregnant rabbits, Waters & Friesen (1979) used both late-pregnant and early-suckling rabbits, whereas Herington & Veith (1977) used non-pregnant female rabbits. Kelly *et al.* (1974) showed that there is an increase in hepatic binding sites during pregnancy due to an increase in somatogenic rather than lactogenic sites.

In view of discrepancies in the values obtained by different workers for the affinity and capacity of hormone binding, we carried out a thorough investigation of Scatchard analysis of the binding data. The result for human somatotropin, suggesting a single class of binding site with an affinity of $1.9 \times 10^9 \text{ M}^{-1}$, which is slightly increased by solubilization to $3.2 \times 10^9 \text{ M}^{-1}$, is consistent with the results of Kelly *et al.* (1974), using membrane-bound receptors, and Herington & Veith (1977) and Tsushima *et al.* (1980), using solubilized receptors, who reported values of 1.5×10^9 , 3.64×10^9 and $3.2 \times 10^9 \text{ M}^{-1}$ respectively. The different values for capacity reported by these groups, ranging from 356 to 1640 fmol/mg of protein, could be due to variations in the rabbits used, as discussed previously.

Scatchard analysis of saturation curves for bovine

somatotropin and ovine prolactin (using membrane-bound receptors) suggested a single class of binding site in each case with affinities of 4.8×10^9 and $6.06 \times 10^9 \text{ M}^{-1}$ respectively. The low capacity for ovine prolactin (187 fmol/mg of protein compared with 1726 and 769 fmol/mg of protein for human and bovine somatotropin respectively) is surprising in view of the ability of ovine prolactin to displace both ^{125}I -labelled human and bovine somatotropin from membrane-bound receptors. A possible explanation is that unlabelled ovine prolactin binds to more than one site, but is affected by iodination in such a way that binding to at least one of these sites is prevented.

The results obtained with the liver receptor preparation examined here suggest that it contains very few specific lactogenic receptors, of the type found in mammary gland. Mammary-gland receptors, prepared from the rabbits that 'provided' the liver used in the present study, bound ^{125}I -labelled ovine prolactin (or human somatotropin). In complete agreement with the results of Shiu & Friesen (1974a), the bound hormone could be displaced by unlabelled lactogenic hormones (ovine prolactin or human somatotropin) but not by unlabelled somatogenic hormones (bovine somatotropin). This contrasts with the situation in liver, where receptor-bound ^{125}I -labelled ovine prolactin (or human somatotropin) can be almost completely displaced by unlabelled bovine somatotropin. This rather low content of specific lactogenic receptor contrasts with the result of Parke & Forsyth (1975), using adult virgin or parous rabbits, and of Waters & Friesen (1979), using late-pregnant or early-suckling rabbits. This discrepancy might be due to the different physiological states of the rabbits used.

The bulk of the binding of human somatotropin to the hepatic receptor preparation used in the present study appears to be due to the presence of one or more receptors that can bind both somatogenic and lactogenic hormones. The physiological significance of such a receptor is not clear. Although the biological actions of somatotropin have not been studied in detail in the rabbit, it seems likely that the growth-promoting action of prolactin is much lower than that of somatotropin, as in other species. Therefore a receptor that mediates the growth-promoting actions of somatotropin would be expected to have a much greater affinity for this hormone than for prolactin. The inability of rabbit prolactin to displace ^{125}I -labelled human somatotropin suggests that the receptor can in fact distinguish between the homologous somatotropin and prolactin. However the fact that the endogenous (rabbit) somatotropin binds with much lower affinity than many other somatotropins casts doubt on the liver receptor as a mediator of the biological actions of the hormone. We have obtained no evidence for a

more specific high-affinity receptor for rabbit somatotropin, as proposed by Hughes (1979); possibly this site is masked by the large number of less specific receptors unless ^{125}I -labelled rabbit somatotropin is used as labelled ligand.

If the bulk of the human somatotropin-binding sites in these rabbit liver preparations are not mediating the specific biological actions of somatotropin, other biological functions for them must be considered. They may be involved in mediating the actions of other hormones of the somatotropin/prolactin family, such as placental lactogen, or modified forms of somatotropin or prolactin. Alternatively they could be involved in mediating the degradation of somatotropin and prolactin, and their removal from the circulation. Although there is no indication from Scatchard analysis that the bulk of the binding sites for human somatotropin fall into more than one class, the very different binding capacities for ^{125}I -labelled human and bovine somatotropin and ovine prolactin suggest that this may be the case, implying further complexities in the interactions between hormones of the somatotropin/prolactin family and these hepatic receptors.

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