Different characteristics of solubilized lactogen receptors from livers of pregnant and non-pregnant female rats

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Receptors specific for lactogenic hormones were solubilized by 1% (v/v) Triton X-100 from the crude particulate membrane fraction of livers of pregnant and non-pregnant female rats and the characteristics of both preparations were compared. Human ^{125}I -labelled somatotropin was used for binding studies of lactogenic hormone. The solubilized receptor retained most of the characteristics noted in the particulate fraction. The binding of human ^{125}I -labelled somatotropin to the solubilized receptor is a saturable process, depending on temperature and time. Scatchard analysis of displacement curves revealed similar affinity constants ranging from 1.02×10^9 to 1.20×10^9 l/mol, while the binding capacity was 4.5 times greater in the pregnant rat livers than in the non-pregnant female rat livers. The receptors for human ^{125}I -labelled somatotropin from livers of non-pregnant and pregnant female rats were equally adsorbed onto a concanavalin-A-Sepharose column and were dissociated from the column with α -methyl-D-glucoside or α -methyl-D-mannoside in the same manner. By gel filtration on Sepharose 6B, however, the molecular sizes of the hepatic receptors were found to be different. The apparent M_r value was approx. 270 000 with a Stokes' radius of 5.0 nm in the non-pregnant female rats and approx. 330 000 with a Stokes' radius of 5.5 nm in the pregnant rats. Furthermore, isoelectric-focusing experiments showed that a major part of the receptor from the non-pregnant female rat livers had a neutral pI (7.0–8.5), whereas that from pregnant-rat livers had an acidic pI (4.2–4.7). These data suggest that the increase in the lactogenic binding capacity in rat liver membranes during pregnancy may be associated with marked changes of the physicochemical properties of the receptors.

Specific receptors for lactogenic hormones have been demonstrated in a number of tissues from several species. These include rat liver and rabbit adrenal gland, liver, mammary gland and ovary (Kelly *et al.*, 1974; Posner *et al.*, 1974), and mouse liver (Posner, 1976a). Previous studies have shown that the number of hepatic lactogen receptors in these animals is much greater in females than in males, and increase further during pregnancy. The biological effects of prolactin on rat liver have been extensively investigated. They comprise an increase in the activity of ornithine decarboxylase (Richards, 1975) and thymidine kinase (Thomson & Richards, 1978) in the liver of prolactin-treated rats, and the stimulation of somatomedin production in perfused rat livers (Francis & Hill, 1975).

Recently, the characteristics of lactogenic hormone binding to pregnant rabbit mammary glands (Shiu & Friesen, 1974a) and female rat livers (Herington *et al.*, 1976a; Silverstein & Richards, 1979) have been studied. Furthermore, a receptor for lactogen from pregnant rabbit mammary glands has been solubilized and characterized (Shiu & Friesen, 1974b).

In the present study, we describe solubilization and characterization of the lactogen receptors from livers of both non-pregnant and pregnant female rats, and demonstrate that the hepatic receptors from non-pregnant and pregnant female rats are quite different in their physicochemical properties.

Materials and methods

Animals

Adult female and 21–23 days pregnant rats of the

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Wistar strain were purchased from Nihon Bio-Sup. Center, Tokyo, Japan. The pregnancies were timed by the supplier, and the day after the evening mating was considered day 1 of pregnancy.

Hormones and chemicals

Human somatotropin (NIH-HS 1652C, 2.0 units/mg), bovine somatotropin (NIH-B 1003A, 1.9 units/mg), ovine somatotropin (NIH-S9, 1.0 unit/mg), ovine prolactin (NIH-PS-12, 35i.u./mg) and human prolactin (NIH-hPr VLS-3, 30i.u./mg) were gifts from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Md, U.S.A.

Bovine serum albumin (fraction V) was purchased from Miles Laboratories, Kankakee, Il, U.S.A. Phospholipase C (*Clostridium perfringens*) and ribonuclease A were purchased from Worthington, Freehold, NJ, U.S.A. Trypsin (bovine pancreas), trypsin inhibitor (soya bean), and deoxyribonuclease (*Clostridium perfringens*) were obtained from Sigma, St. Louis, Mo, U.S.A. Poly(ethylene glycol) 6000 was purchased from Nakarai Chemicals, Tokyo, Japan and Triton X-100 was a product of Eastman Organic Chemicals, New York, NY, U.S.A. Carrier-free Na¹²⁵I was purchased from New England Nuclear, Boston, Ma, U.S.A. Sephadex and concanavalin-A-Sepharose were purchased from Pharmacia, Uppsala, Sweden.

Iodination of human somatotropin

Ovine ¹²⁵I-labelled prolactin has been reported to be unsuitable as the tracer in binding studies on solubilized lactogen receptor because of the formation of large complexes and loss of binding activity in the presence of detergent (Triton X-100) (Shiu & Friesen, 1974b). In contrast, human ¹²⁵I-labelled somatotropin has been shown to be stable in the detergent and human somatotropin is known to act as a lactogenic hormone in rats. Therefore, in the present study, human ¹²⁵I-labelled somatotropin was used as the tracer. Human somatotropin was iodinated by the chloramine-T method (Greenwood *et al.*, 1963) modified as follows. Human somatotropin (5 µg), 0.5 M-phosphate buffer (pH 7.4) (20 µl), Na¹²⁵I (0.5 mCi) and chloramine-T (10 µg) were added sequentially and allowed to react for 60 s. The iodination reaction was terminated by the addition of 10 µg of sodium metabisulphite. The labelled hormone was separated from unreacted iodine and damaged aggregate by chromatography on a column (1 cm × 40 cm) of Sephadex G-100 in 50 mM-phosphate buffer, pH 7.4. The specific activity was 80–100 µCi/µg.

Solubilization of the lactogen receptors

The crude membrane fractions containing lactogen receptors were prepared from livers of

non-pregnant and pregnant female rats as described previously (Tsushima & Friesen, 1973; Tsushima *et al.*, 1980). The resultant 100 000 g pellets, which are referred to as particulate receptors, were solubilized according to Shiu & Friesen (1974b). The 100 000 g pellets were suspended and homogenized in 50 mM-Tris/HCl buffer, pH 7.4. The protein concentrations of the suspensions were adjusted to 5 mg/ml and Triton X-100 was added to a final concentration of 1% (v/v). The mixtures were stirred for 1 h at 25°C, and then centrifuged at 100 000 g for 2 h at 4°C. The supernatants were stored at –20°C until use.

Binding studies

Particulate membrane receptors. The particulate fractions (50–200 µg of protein/tube) were incubated with human ¹²⁵I-labelled somatotropin (50 000–70 000 c.p.m.) in a final volume of 0.5 ml of 50 mM-Tris/HCl buffer, pH 7.4, containing 0.1% bovine serum albumin and 10 mM-MgCl₂. All incubations were carried out in glass tubes (1.0 cm × 7.5 cm) at room temperature (21–25°C) for 3 h. After the termination of the reaction by adding 2 ml of ice-cold buffer, bound and free human ¹²⁵I-labelled somatotropin were separated by centrifugation at 1000 g for 30 min at 4°C. The supernatant was removed and the pellet was counted in a gamma counter. In all studies, specific binding was calculated by subtracting the bound non-specific hormone from the bound total labelled hormone. Bound non-specific hormone is that which is bound in the presence of an excess of unlabelled hormone (1 µg/tube).

Solubilized receptors. Solubilized receptor was incubated in the same conditions as described above. Poly(ethylene glycol) 6000 [final concentration 12.5% (w/v)] was used to precipitate the bound hormone as described (Cuatrecasas, 1972a; Shiu & Friesen, 1974b). In our assay conditions, final concentrations of Triton X-100 greater than 0.05% (v/v) were found to inhibit human somatotropin binding. Therefore, Triton extracts of membranes were diluted so that the final concentration of the detergent was less than 0.05%, and usually less than 0.02%. To estimate the efficacy of the separation of bound and free hormone by poly(ethylene glycol), gel filtration was used in some experiments. A 0.1 ml aliquot containing solubilized human somatotropin receptors was incubated with human ¹²⁵I-labelled somatotropin in the presence or absence of excess unlabelled somatotropin (1 µg/tube). At the end of the incubation, the total incubation mixture (1 ml) was fractionated at 4°C on a Sephadex G-100 column (1.5 cm × 35 cm) previously equilibrated with 50 mM-Tris/HCl buffer, pH 7.4, containing 0.1% (v/v) Triton X-100. Fractions (0.6 ml) were collected and the radioactivity of aliquots was measured in a gamma counter.

Affinity chromatography

Each Triton extract (10 ml) from the 100 000 g pellets of livers of non-pregnant and pregnant female rats was diluted with an equal volume of 50 mM-Tris/HCl buffer, pH 7.4, containing 0.1% (v/v) Triton X-100. Each was chromatographed at 4°C on a column (2 cm × 6.4 cm) packed with concanavalin-A-Sepharose that had been extensively washed with the same buffer. The flow rate was adjusted to 20 ml/h and fractions (4 ml) were collected. The column was washed with the buffer until no protein was eluted. Elution of human somatotropin-binding activity was achieved with 2 bed volumes of 0.1 M- α -methyl-D-glucoside in the buffer. After extensive washing of the column with the buffer, a second elution was performed using 0.1 M- α -methyl-D-mannoside in the buffer. Each fraction was determined for human somatotropin-binding activities and protein concentrations. Protein concentrations in the eluates were determined by the method of Lowry *et al.* (1951). Because high concentrations of Tris (over 0.02 M) or Triton X-100 (over 0.1%) may interfere with the Lowry assay, blanks were run in the same buffer.

Molecular size determination

A column of Sepharose 6B (1 cm × 100 cm) equilibrated with 50 mM-Tris/HCl buffer, pH 7.4, containing 0.1% Triton X-100 was used to determine the molecular sizes of human somatotropin receptors from liver membranes of non-pregnant and pregnant female rats. The respective preparation was applied to the column and chromatographed at a flow rate of 4 ml/h at 4°C. Fractions (1.3 ml) were collected and aliquots were taken for the determination of the binding activity for human ^{125}I -labelled somatotropin. The column was calibrated with several marker proteins including apoferritin, bovine gamma globulin, bovine serum albumin, ovalbumin and human ^{125}I -labelled somatotropin. The void volume (V_0) and the total liquid volume (V_t) were determined by the elution of Blue Dextran 2000 (Pharmacia) and Na^{125}I , respectively. The chromatographic behaviour was expressed in terms of K_{av} , according to Laurent & Killander (1964) as:

$$K_{av} = (V_e - V_0)/(V_t - V_0)$$

where V_e is the elution volume of the protein. The Stokes' radius was calculated according to Siegel & Monty (1966).

Isoelectric focusing

Isoelectric focusings between pH 3.5 and pH 10.0 were conducted on preparative flat-bed gels of granulated gel in an LKB 2117 Multiphor apparatus (LKB Produkter AB, Sweden), according to LKB application note 198. Each Triton X-100-solubilized extract (2 ml) from livers of non-pregnant and

pregnant female rats was diluted to 95 ml with distilled water, and 5 ml of Ampholine carrier ampholytes (LKB, 1809-101, pH 3.5–10.0) were added. The concentration of Triton X-100 was adjusted to 0.1% (v/v). Sephadex G-75 superfine (5 g; Ultradex, LKB) was slowly added to the mixture. Then the suspension was poured onto a glass plate and placed in the Multiphor. Focusing was carried out at 4°C for 12 h at a constant power of 8 W. At the end of electrophoresis the gel bed was sectioned into 30 fractions with a grid, and each gel segment was transferred to a small empty column by using a spatula. The column was then eluted with 2 ml of distilled water. The eluate was assayed for binding activity to human ^{125}I -labelled somatotropin.

Results

Solubilization

Of the fraction from pregnant rat livers, 74% of the total protein of the initial 100 000 g pellet and 90% of the binding capacity for human ^{125}I -labelled somatotropin, as judged by the analysis of Scatchard (1949), were solubilized with 1% Triton X-100 and remained in the final supernatant. Similarly, 81% of the protein and 95% of the binding capacity were recovered in the solubilized fraction from non-pregnant female rat livers.

Binding studies

The Triton-solubilized extract from pregnant rat livers was incubated with human ^{125}I -labelled somatotropin in the presence or absence of unlabelled human somatotropin, and the reaction mixture was subjected to gel filtration (Fig. 1). In the absence of unlabelled human somatotropin, 60% of the total radioactivity was detected in the void-volume fractions. On the other hand, in the presence of excess human somatotropin most of the labelled hormone eluted with proteins of M_r 20 000, indicating that human ^{125}I -labelled somatotropin had been almost completely displaced from the large complex. The solubilized fraction from non-pregnant female rat livers gave a similar gel filtration pattern (results not shown).

Binding activity in Triton-solubilized extract was also demonstrated by using poly(ethylene glycol) precipitation. Non-specific precipitation of unbound human ^{125}I -labelled somatotropin was less than 5% of the total radioactivity. The extent of specific binding obtained by the poly(ethylene glycol) assay was in good agreement with that determined by gel filtration on Sephadex G-100, and hence the former method was employed in the study because of its simplicity. Specific binding of human ^{125}I -labelled somatotropin to the solubilized receptors from livers of both pregnant and non-pregnant female rats

Fig. 1. Gel filtration pattern demonstrating specific binding of human ^{125}I -labelled somatotropin to the solubilized 100 000 g pellet of liver of pregnant rat. Approx. 150 000 c.p.m. of human ^{125}I -labelled somatotropin was incubated with Triton extract (500 μg of protein) in 1 ml of 50 mM-Tris/HCl buffer, pH 7.4, containing 0.1% bovine serum albumin and 10 mM- MgCl_2 at 25°C for 3 h in the presence (O) or absence (●) of 1 μg /tube of unlabelled human somatotropin. The incubation mixture was applied to a column (1.5 cm $\times$ 35 cm) of Sephadex G-100, and the radioactivity of 0.6 ml fractions was measured.

increased equally in a linear manner with protein concentrations of up to 100 μg /tube.

The time course of binding of human ^{125}I -labelled somatotropin to the solubilized liver receptor from pregnant rats, which was similar to that obtained from non-pregnant female rat liver, is shown in Fig. 2. As shown, at 25°C , a steady state was attained within 3 h, whereas binding at 4°C was very slow.

Specificity of the receptors

The specificity of the solubilized receptors from livers of non-pregnant and pregnant female rats is shown in Fig. 3. The Figure shows that increasing amounts of unlabelled human somatotropin dis-

Fig. 2. Effect of time and temperature on the binding of human ^{125}I -labelled somatotropin by the solubilized receptor from livers of pregnant rats

Triton extract (50 μg of protein) was incubated with 50 000 c.p.m. of human ^{125}I -labelled somatotropin at 4°C or 25°C , and the specific binding of human ^{125}I -labelled somatotropin (% of total radioactivity) was calculated. Δ , 4°C ; O, 25°C .

placed the labelled hormone from the receptor. As observed in the previous studies using particulate membrane fractions (Herington *et al.*, 1976a, b; Posner, 1976b), unlabelled ovine prolactin and human prolactin displaced human ^{125}I -labelled somatotropin in a similar manner. Somatotropins from sheep and ox were without effect except at very high concentrations. The binding specificity of the solubilized fractions from non-pregnant female (Fig. 3a) and pregnant (Fig. 3b) rat livers was almost identical.

Scatchard analysis of human ^{125}I -labelled somatotropin binding

Scatchard analyses (Scatchard, 1949) of the data from dose-response curves of the displacement of human ^{125}I -labelled somatotropin binding were carried out on both the particulate and the solubilized fractions from livers of non-pregnant and pregnant female rats (Fig. 4). They indicated that each fraction has a single type of binding site. In non-pregnant female preparations (Fig. 4a), binding capacity was increased (600 ± 25 fmol/mg of protein to 1010 ± 30 fmol/mg of protein; mean $\pm$ s.d. of four experiments) by solubilization, although binding affinity was not affected [$(1.17 \pm 0.04) \times 10^9$ l/mol versus $(1.20 \pm 0.05) \times 10^9$ l/mol]. Similarly, in the pregnant preparations (Fig. 4b), binding capacity was augmented (1800 ± 55 to 4480 ± 90 fmol/mg of protein) without change in the binding affinity

Fig. 3. Hormonal specificity for the binding of human ^{125}I -labelled somatotropin to the solubilized receptors from livers of non-pregnant (a) and pregnant (b) female rats

The specific binding of human ^{125}I -labelled somatotropin was plotted as a percentage of that obtained in the absence of unlabelled hormone. Protein from the Triton extract ($50\mu\text{g}$) was incubated with 50000 c.p.m. of human ^{125}I -labelled somatotropin in the presence of increasing amounts of human somatotropin (●), ovine prolactin (○), human prolactin (△), or somatotropins from sheep (□) and ox (■).

$[(1.00 \pm 0.02) \times 10^9/\text{mol}$ to $(1.02 \pm 0.03) \times 10^9/\text{mol}]$. The values for the binding affinity in the particulate fraction were comparable to those reported by Herington *et al.* (1976b), but the values for the binding capacity were greater than those described in the previous work. The discrepancy may be due to the difference in the strain of the rat from which liver membrane fractions were prepared (Wistar versus Sprague-Dawley). As regards the solubilized preparations, binding capacity was much greater in pregnant rat livers than in those of non-pregnant female, whereas binding affinity did not differ.

Affinity chromatography

As shown in Fig. 5, most of the protein in the solubilized fraction from pregnant-rat livers passed through the column. Of the total binding activity adsorbed on to the column, approx. 60% was recovered in the fractions eluted with 0.1 M- α -methyl-D-glucoside, and the remaining binding activity was obtained by further washing the column with 0.1 M- α -methyl-D-mannoside (Fig. 5a). When the elution order of glucoside and mannoside was reversed, most of the binding activity was recovered in the fractions eluted by mannoside, and the remnant was obtained in those eluted by glucoside (Fig. 5b).

The elution profiles of Triton extract from non-pregnant female rat livers on the affinity chromatography column were almost identical to the profiles obtained with pregnant-rat livers.

Gel filtration

Fig. 6 indicates the elution pattern for the Triton extracts from livers of pregnant (Fig. 6a) and non-pregnant (Fig. 6b) female rats on a Sepharose 6B column. As shown in the Figure, the binding activity for human ^{125}I -labelled somatotropin from livers of pregnant rats was eluted earlier than that from livers of non-pregnant female rats. The K_{av} values of receptors for human somatotropin from livers of pregnant and non-pregnant female rats were 0.35 ± 0.02 (mean $\pm$ s.d. for six experiments) and 0.40 ± 0.02 (mean $\pm$ s.d. for six experiments), respectively. Based on those values, the M_r values of the receptors from livers of pregnant and non-pregnant female rats were estimated to be $334\,700 \pm 33\,200$ (mean $\pm$ s.d.) and $269\,200 \pm 26\,900$ respectively, assuming that the receptors and marker proteins have the same symmetry (Fig. 7). The Stokes' radii of the receptors from livers of pregnant and non-pregnant female rats were calculated to be $5.5 \pm 0.2\text{ nm}$ (mean $\pm$ s.d.) and $5.0 \pm 0.2\text{ nm}$, respectively. These differences in M_r values and Stokes' radii were statistically significant ($P < 0.01$).

Fig. 4. Scatchard plots of the data from displacement curves of human ^{125}I -labelled somatotropin binding to liver fractions (particulate, $\circ$; solubilized, $\bullet$) from non-pregnant (a) and pregnant (b) female rats

Each fraction ($50\mu\text{g}$ of protein) was incubated with 50000 c.p.m. of human ^{125}I -labelled somatotropin for 3 h at 25°C . The ordinate represents the ratio of bound/free, and the abscissa the amount (fmol) of human somatotropin bound to rat liver membranes.

Isoelectric focusing

Each Triton extract from livers of pregnant and non-pregnant female rats was subjected to isoelectric focusing between pH 3.5 and 10.0 in a flat-bed gel (Fig. 8). The binding activity for human ^{125}I -labelled somatotropin from pregnant rat livers was mainly focused between pH 4.0 and pH 5.4, with peak activity at pH 4.2–4.7 ($72 \pm 5\%$ of total activity; mean $\pm$ S.D. for three experiments), and another small peak between pH 6.5 and 7.3 (Fig. 8a). In contrast, the binding activity from non-pregnant female rat livers was broadly distributed between pH 6.3 and pH 9.4, with major activity at

Fig. 5. Affinity chromatography on concanavalin-A-Sepharose

Triton extract (10 ml) from pregnant rat livers was diluted with an equal volume of 50 mM -Tris/HCl buffer, pH 7.4, containing 0.1% (v/v) Triton X-100. It was chromatographed on a column ($2\text{ cm} \times 6.4\text{ cm}$) of concanavalin-A-Sepharose. The column was washed with the buffer until no protein was eluted, then was eluted in the order 0.1 M - α -methyl-D-glucoside and 0.1 M - α -methyl-D-mannoside (a) or in the reverse order (b). The specific binding of human ^{125}I -labelled somatotropin ($\bullet$) was determined by using $100\mu\text{l}$ of the 4 ml fractions and 70000 c.p.m. of labelled hormone. Protein concentrations ($\circ$) were also determined by the method of Lowry *et al.* (1951).

pH 7.0–8.5 ($51 \pm 6\%$ of total activity; mean $\pm$ S.D. for three experiments), the acidic portion (pH 3.9–5.0) not being prominent (Fig. 8b). The proportion of the binding activity focussed at pH 7.0–8.5 from pregnant rat livers was constantly less than 1% of total activity, and the ratio of the activity at pH 4.2–4.7 from non-pregnant rat livers was $7 \pm 2\%$ of total activity.

Effect of enzymes

Treatment of solubilized receptors from livers of either non-pregnant or pregnant female rats with trypsin resulted in a significant loss of the binding activity for human ^{125}I -labelled somatotropin (Table 1). Ribonuclease, deoxyribonuclease and phospholipase C did not affect the binding activity. Higher concentrations of neuraminidase increased the binding activity slightly in both preparations. Isoelectric

Fig. 6. Gel filtration on a Sepharose 6B column. Each Triton extract (0.5 ml) from livers of pregnant (a) or non-pregnant (b) female rats was filtered on a Sepharose 6B column (1 cm $\times$ 100 cm) equilibrated with 50 mM-Tris/HCl buffer, pH 7.4, containing 0.1% Triton X-100. Chromatography was carried out at a flow rate of 4 ml/h at 4°C. Fractions (1.3 ml) were collected and the binding activity for human ^{125}I -labelled somatotropin (●) was determined by using 100 μl of the eluate and 50 000 c.p.m. of labelled hormone. Protein concentrations (○) were also determined by the method of Lowry *et al.* (1951). V_0 , void volume; V_t , bed volume of the column.

focusing of pregnant preparation treated with neuraminidase (80 $\mu\text{g/ml}$) showed that the peak of binding activity shifted from pH 4.2–4.7 to pH 4.9–5.3 (results not shown). In contrast, treatment of non-pregnant female preparations with neuraminidase (80 $\mu\text{g/ml}$) did not affect the peak of binding activity in isoelectric focusing. The M_r values determined by use of the Sepharose 6B column did not differ after the treatment of the preparations with neuraminidase.

Discussion

Receptors for a variety of polypeptide hormones have been solubilized and characterized (Dufau *et al.*, 1973; Jacobs *et al.*, 1977; Waters & Friesen, 1979; Tsushima *et al.*, 1980). Shiu & Friesen (1974b) have described the solubilization of lactogen

Fig. 7. Estimation of approximate M_r value of human somatotropin receptors by Sepharose 6B gel filtration. The K_{av} values of human somatotropin receptors (pregnant, $\square$; non-pregnant female, Δ) and the marker proteins were determined from the chromatographic behaviour of the proteins on the Sepharose 6B column used in Fig. 6. Each marker protein was dissolved in 0.5 ml of 50 mM-Tris/HCl buffer, pH 7.4, containing 0.1% Triton X-100, and was filtered separately on the column. Fractions (1.3 ml) were collected and protein concentrations were determined by the method of Lowry *et al.* (1951).

receptor from pregnant rabbit mammary gland by using the non-ionic detergent Triton X-100. However, the properties of the lactogen receptor from rat liver have not yet been reported. In the present study, the hepatic receptors for lactogenic hormone from non-pregnant and pregnant female rats were solubilized with 1% (v/v) Triton X-100 and characterized. Because of the stability and lactogenic activity, human ^{125}I -labelled somatotropin was used as the tracer in the binding study.

In common with the receptors for other hormones, the solubilized human somatotropin receptor emerged as a large molecule on gel filtration on a Sephadex G-100 column. The binding of human ^{125}I -labelled somatotropin was rapid, saturable, dependent on time and temperature, and increased as a function of protein concentration. The binding affinity constants for human somatotropin of preparations of non-pregnant and pregnant female rats were not affected by solubilization. The result is different from that reported by Shiu & Friesen (1974b) in that lactogen receptor, when solubilized

Fig. 8. *Isoelectric focusing*

Isoelectric focusing of the Triton extract (10 mg of protein) from livers of pregnant (a) or non-pregnant (b) female rats was performed as described in the Materials and methods section. The eluate from each gel section was incubated with 60 000 c.p.m. of human ^{125}I -labelled somatotropin in 0.5 ml of 50 mM-Tris/HCl buffer, pH 7.4, containing 0.1% bovine serum albumin and 10 mM- MgCl_2 , and the binding activity was determined. Bars indicate specific binding of human ^{125}I -labelled somatotropin. ●, pH.

Table 1. *Effect of enzymes on the specific binding of human ^{125}I -labelled somatotropin to the solubilized receptors from livers of non-pregnant and pregnant female rats*

Solubilized receptors (50 μg of protein) were incubated with different concentrations of enzymes at 37°C for 20 min in 50 mM-Tris/HCl buffer, pH 7.4. For trypsin digestion of the preparations, a 5-fold excess (over trypsin) of soya bean trypsin inhibitor was added immediately after the treatment. The treated medium (0.1 ml) was incubated with 50 000 c.p.m. of human ^{125}I -labelled somatotropin at 25°C for 3 h to determine the binding activity. For controls, fractions that were not exposed to enzymes but incubated in the same buffer for the same periods were used. The experiment was carried out in duplicate and the value is the mean of duplicate determinations.

Enzyme	Concentration ($\mu\text{g}/\text{ml}$)	Specific binding of human ^{125}I -labelled somatotropin (% of control)	
		Non-pregnant	Pregnant
Trypsin	80	5	3
	10	43	38
Ribonuclease	80	96	92
	10	98	95
Deoxyribonuclease	80	94	88
	10	89	93
Phospholipase C	80	87	83
	10	86	91
Neuraminidase	80	115	123
	10	97	93

with Triton, exhibits a 5-fold increase in its affinity. It is difficult to provide an explanation for the discrepancy. The specificity of binding of human ^{125}I -labelled somatotropin is similar to that observed with the particulate fraction (Herington *et al.*, 1976a,b; Posner, 1976b). These results suggest that extraction of the receptor with Triton X-100 does not cause any major changes in properties of the molecules.

Recently, several hormone receptors have been partially purified by using affinity chromatography (Cuatrecasas & Tell, 1973; Shiu & Friesen, 1974b; Dufau *et al.*, 1975; Jacobs *et al.*, 1977; Waters & Friesen, 1979; Tsushima *et al.*, 1980). Concanavalin A has been shown to inhibit prolactin binding to rat liver membrane (Costlow & Gallagher, 1977). Like the insulin receptor from rat liver (Cuatrecasas & Tell, 1973; Jacobs *et al.*, 1977) and the somatotropin receptor from rabbit liver (Tsushima *et al.*, 1980), the receptors for human ^{125}I -labelled somato-

trophin from livers of non-pregnant and pregnant female rats were equally adsorbed onto the concanavalin-A-Sepharose column, and they were effectively eluted from the column by either α -methyl-D-glucoside or α -methyl-D-mannoside. This finding strongly suggests that the receptors from livers of non-pregnant and pregnant female rats may be glycoproteins.

By gel filtration on Sepharose 6B, the M_r values of the hepatic receptors from non-pregnant and pregnant female rats were estimated to be approx. 270 000 with a Stokes' radius of 5.0 nm, and 330 000 with a Stokes' radius of 5.5 nm, respectively. The difference in molecular size was statistically significant. The apparent M_r value (220 000) of the prolactin receptor from rabbit mammary gland (Shiu & Friesen, 1974b) is slightly lower than the values we have found. The values for M_r and the Stokes' radius we have found are similar to those

reported for gonadotropin (Dufau *et al.*, 1973), insulin (Cuatrecasas, 1972b; Jacobs *et al.*, 1977) and somatotropin receptors (Waters & Friesen, 1979; Tsushima *et al.*, 1980). Isoelectric-focusing experiments revealed that the hepatic receptor from non-pregnant female rats has a neutral pI, and that from pregnant rats an acidic pI. The acidic pI of the lactogen receptor from pregnant rat livers is comparable with that of the insulin receptor (Jacobs *et al.*, 1977) or the somatotropin receptor (Waters & Friesen, 1979; Tsushima *et al.*, 1980).

Treatment of the receptors from either non-pregnant or pregnant female rats with trypsin resulted in a significant loss of the binding activity, indicating that in both preparations polypeptide forms an important component of the receptor. The acidic pI of the lactogen receptor from pregnant rats was increased by neuraminidase treatment, as reported for the insulin receptor (Kasuga *et al.*, 1980). The change of the pI seems to be due to a loss of negatively charged sialic acid residues. The neutral pI of the receptor from non-pregnant female rats was not affected. The sialic acid content of the receptor from non-pregnant female rats may be less than that from pregnant rats. Treatment of both receptors with neuraminidase similarly led to slight increases in binding activity. It is not clear whether sialic acid is involved in the binding reaction or not. Thus, the lactogen receptors from livers of non-pregnant and pregnant female rats differ markedly in some properties, although both types of lactogen receptors seem to share several characteristics with receptors for other polypeptide hormones.

It is generally accepted that receptors of various hormones alter in their capacities and affinities under numerous physiological conditions. As for the prolactin receptor, previous studies have shown that the number of lactogen receptors in liver membrane fractions is much greater in female rats, and that it increases further in pregnancy (Kelly *et al.*, 1974; Posner *et al.*, 1974). The increase in the binding capacity was attributed to the increase in the number of receptors. However, the changes in the apparent molecular size or isoelectric point of a receptor molecule have not been reported before.

The altered physicochemical properties, such as apparent molecular size and isoelectric point, of the glycoprotein hormones in various physiological and pathological conditions have been well documented (Peckham *et al.*, 1973; Bogdanove *et al.*, 1974; Peckham & Knobil, 1976a,b; Klug & Adelman, 1977; Benveniste *et al.*, 1979). Although the biological significance of these physicochemical changes of glycoprotein hormones is unclear, the heterogeneity of the glycoproteins in these several states has been shown to be due to, in large part, alterations in the carbohydrate portion of the molecules and occur in the context of the increased

production of the hormones (Peckham & Knobil, 1976a,b; Fein *et al.*, 1980).

Since the lactogen receptors are suggested to be glycoproteins and the production of the receptors is apparently increased during pregnancy, the differences in the physicochemical characteristics of the lactogen receptors may be considered as analogous to the heterogeneity of glycoprotein hormones. As for glycoprotein hormones, the biological significance of the heterogeneity is not clear at present, and awaits further studies for elucidation.

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References

- Benveniste, R., Conway, M. C., Puett, D. & Rabinowitz, D. (1979) *J. Clin. Endocrinol. Metab.* **48**, 85–91
- Bogdanove, E. M., Campbell, G. T. & Peckham, W. D. (1974) *Endocrine Res. Commun.* **1**, 87–99
- Costlow, M. E. & Gallagher, P. E. (1977) *Biochem. Biophys. Res. Commun.* **77**, 905–911
- Cuatrecasas, P. (1972a) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 318–322
- Cuatrecasas, P. (1972b) *J. Biol. Chem.* **247**, 1980–1991
- Cuatrecasas, P. & Tell, G. P. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 485–489
- Dufau, M. L., Charreau, E. H. & Catt, K. J. (1973) *J. Biol. Chem.* **248**, 6973–6982
- Dufau, M. L., Ryan, D. W., Baukal, A. J. & Catt, K. J. (1975) *J. Biol. Chem.* **250**, 4822–4824
- Fein, H. G., Rosen, S. W. & Weintraub, B. D. (1980) *J. Clin. Endocrinol. Metab.* **50**, 1111–1120
- Francis, M. J. O. & Hill, D. J. (1975) *Nature (London)* **255**, 167–168
- Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) *Biochem. J.* **89**, 114–123
- Herington, A. C., Veith, N. & Burger, H. G. (1976a) *Biochem. J.* **158**, 61–69
- Herington, A. C., Phillips, L. S. & Daughaday, W. H. (1976b) *Metabolism* **25**, 341–353
- Jacobs, S., Schechter, Y., Bissell, K. & Cuatrecasas, P. (1977) *Biochem. Biophys. Res. Commun.* **77**, 981–987
- Kasuga, M., Ezaki, O., Akanuma, Y. & Kosaka, K. (1980) *Horm. Metab. Res.* **12**, 494–496
- Kelly, P. A., Posner, B. I., Tsushima, T. & Friesen, H. G. (1974) *Endocrinology* **95**, 532–539
- Klug, T. L. & Adelman, R. C. (1977) *Biochem. Biophys. Res. Commun.* **77**, 1431–1437
- Laurent, T. C. & Killander, J. (1964) *J. Chromatogr.* **14**, 317–330
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Peckham, W. D. & Knobil, E. (1976a) *Endocrinology* **98**, 1054–1060
- Peckham, W. D. & Knobil, E. (1976b) *Endocrinology* **98**, 1061–1064
- Peckham, W. D., Yamaji, T., Dierschke, D. J. & Knobil, E. (1973) *Endocrinology* **92**, 1660–1666

- Posner, B. I. (1976a) *Endocrinology* **98**, 645–654
- Posner, B. I. (1976b) *Endocrinology* **99**, 1168–1177
- Posner, B. I., Kelly, P. A., Shiu, R. P. C. & Friesen, H. G. (1974) *Endocrinology* **95**, 521–531
- Richards, J. F. (1975) *Biochem. Biophys. Res. Commun.* **63**, 292–299
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Shiu, R. P. C. & Friesen, H. G. (1974a) *Biochem. J.* **140**, 301–311
- Shiu, R. P. C. & Friesen, H. G. (1974b) *J. Biol. Chem.* **240**, 7902–7911
- Siegel, L. M. & Monty, K. J. (1966) *Biochim. Biophys. Acta* **112**, 246–362
- Silverstein, A. M. & Richards, J. F. (1979) *Biochem. J.* **178**, 743–751
- Thomson, M. J. & Richards, J. F. (1978) *Life Sci.* **22**, 337–344
- Tsushima, T. & Friesen, H. G. (1973) *J. Clin. Endocrinol. Metab.* **37**, 334–337
- Tsushima, T., Sasaki, N., Imai, Y., Matsuzaki, F. & Friesen, H. G. (1980) *Biochem. J.* **187**, 479–492
- Waters, M. J. & Friesen, H. G. (1979) *J. Biol. Chem.* **254**, 6815–6825