The hormone-binding unit of luteinizing hormone receptor

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Rat ovarian luteinizing hormone/human choriogonadotropin binding sites were labelled with ^{125}I -choriogonadotropin *in vivo*, and the resulting ^{125}I -choriogonadotropin–receptor complexes were solubilized by Triton X-100 and purified by use of antibodies to choriogonadotropin immobilized to agarose. The purified ^{125}I -choriogonadotropin–receptor complex was treated with glutaraldehyde to crosslink radiolabelled hormone to the receptor. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the crosslinked product revealed a labelled M_r 130 000 major band in addition to the hormone and its α -subunit, indicating that a single receptor component was linked to the hormone. Unoccupied binding sites for luteinizing hormone were also solubilized by Triton X-100 from pseudopregnant rat ovaries, and attached to choriogonadotropin–agarose. The agarose gel was washed, and eluted with 0.1 M-sodium acetate, pH 4. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the pH 4 eluate revealed an M_r 90 000 major band which was abolished when ovaries presaturated with choriogonadotropin were used as starting material. These observations suggest that the hormone-binding component of the luteinizing hormone receptor is a polypeptide of M_r 90 000. This polypeptide was isolated and labelled with Na^{125}I . The labelled polypeptide showed a single band on sucrose density gradient centrifugation and on gel filtration on agarose.

Luteinizing hormone receptor has been solubilized from rat ovary and testis and its M_r was determined to be about 200 000 (Dufau *et al.*, 1973; Dufau & Catt, 1976). Several attempts to purify and characterize the receptor have been published. A 15 000-fold purification of rat testicular receptor by affinity chromatography has been reported (Dufau *et al.*, 1975). The receptor has been identified and its properties characterized by means of radiolabelling of bovine corpus luteum plasma membranes (Pandian & Bahl, 1977). An M_r 110 000 receptor polypeptide has been purified from rat ovary by use of immobilized hormone antibodies (Metsikkö & Rajaniemi, 1980), while a covalent affinity cross-linking method has revealed an M_r 100 000 choriogonadotropin-binding polypeptide (Rebois *et al.*, 1981). However, porcine granulosa cells have been shown to produce three or four bands in the M_r 63 000–120 000 range when analysed by SDS/polyacrylamide-gel electrophoresis after photo-affinity crosslinking to choriogonadotropin (Ji & Ji, 1980; Ji *et al.*, 1981). Hence, the hormone-binding component of the luteinizing hormone receptor has not been identified with certainty, and the overall

subunit composition of the receptor remains uncertain.

In the present study we have crosslinked purified ^{125}I -choriogonadotropin–receptor complex by glutaraldehyde, when a single major receptor component became attached to the hormone. On the other hand, affinity chromatography produced a component which revealed an M_r 90 000 polypeptide when subjected to SDS/polyacrylamide-gel electrophoresis. This component was not obtained when the starting material was saturated by hormone.

These data show that the hormone-binding component of the luteinizing hormone/choriogonadotropin receptor is an M_r 90 000 polypeptide. This polypeptide was isolated and labelled with ^{125}I .

Experimental

Chemicals

Pregnant mare serum gonadotropin (1500 units/mg) and human choriogonadotropin (10 000 units/mg) were obtained from Diosynth, Oss, Holland. CNBr-activated Sepharose 4B, Sephacryl S-300, Sephadex G-25 and Blue Dextran were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden.

Abbreviations used: SDS, sodium dodecyl sulphate.

Affigel 10 was obtained from Bio-Rad Chemicals, Richmond, CA, U.S.A. Na¹²⁵I was obtained from the Radiochemical Centre, Amersham, Bucks., U.K., and albumin, myosin, ovalbumin, and phosphorylase b from Sigma Chemical Co., St. Louis, MO, U.S.A. All other reagents were of analytical grade and obtained from commercial suppliers.

Animals

Female 27-day-old Wistar rats were induced pseudopregnant by treatment with pregnant mare serum gonadotropin and choriogonadotropin as described by Parlow (1961). The animals were used 7 days after the choriogonadotropin injection.

Purification of ¹²⁵I-choriogonadotropin-receptor complex

¹²⁵I-choriogonadotropin, prepared according to Markkanen *et al.* (1980), was injected into the tail vein of pseudopregnant rats (10⁷ c.p.m. of ¹²⁵I-choriogonadotropin; 200 µl). The animals were sacrificed 2 h later, and the ovaries were homogenized in phosphate-buffered saline (10 mM-sodium phosphate, pH 7.0, containing 140 mM-NaCl) (1 ovary/ml), and centrifuged for 30 min at 20 000 g at 4°C. The pellet was solubilized by Triton X-100 (Dufau *et al.*, 1974) and the ¹²⁵I-choriogonadotropin-receptor complex was purified by means of anti-choriogonadotropin-Sepharose 4B as described earlier (Metsikkö & Rajaniemi, 1979).

Glutaraldehyde treatment

The purified ¹²⁵I-choriogonadotropin-receptor complex obtained by displacement with choriogonadotropin from the anti-choriogonadotropin-Sepharose 4B (Metsikkö & Rajaniemi, 1979) was incubated with 0.5 mM-glutaraldehyde by adding 25 µl of 5 mM-glutaraldehyde, pH 7.0, to 225 µl of purified ¹²⁵I-choriogonadotropin-receptor complex (250 000 c.p.m.). The solution was incubated for 4 h at 20°C, pH 7.0, and dialysed into 125 mM-Tris/HCl, pH 7.0, containing 0.1% Triton X-100, for 24 h at 4°C. For a control, ¹²⁵I-choriogonadotropin was attached to anti-choriogonadotropin-Sepharose 4B, displaced by unlabelled choriogonadotropin and treated with glutaraldehyde identically to the sample.

Affinity chromatography

Human choriogonadotropin was coupled to Affigel 10 to purify the luteinizing hormone receptor. In detail, 1 mg of choriogonadotropin was dissolved in 1.0 ml of 0.1 M-NaHCO₃, pH 7.5 (adjusted with 1 M-HCl), containing 0.5 M-NaCl, and agitated with 1 ml of Affigel 10 beads for 4 h at 4°C in a sealed syringe. The remaining active groups were blocked by adding 0.1 ml of 1 M-ethanolamine, pH 8.0 for 1 h at 20°C. The gel beads were packed into a column and washed with 10 ml of 0.1 M-

sodium acetate, pH 4.0, containing 0.5 M-NaCl, and with 10 ml of 0.1 M-sodium phosphate, pH 9.0, containing 0.5 M-NaCl, four times successively. Later, 0.1 M-Tris/HCl, pH 9.0, containing 0.5 M-NaCl was used instead of the phosphate buffer. The gel was finally washed with 20 ml of phosphate-buffered saline containing 0.5% Triton X-100. The coupling efficiency was 60%. About 100 µl of the choriogonadotropin-Affigel 10 was able to bind receptors solubilized from 1 g of ovarian tissue.

Pseudopregnant rat ovarian tissue (3 g from 10–15 rats) was homogenized in a glass homogenizer in 30 ml of phosphate-buffered saline at 0°C, and centrifuged for 30 min at 20 000 g at 4°C. The pellet was suspended in 15 ml of phosphate-buffered saline containing 1% (w/v) Triton X-100, 1 mM-phenylmethanesulphonyl fluoride and, when indicated, 10 mM-iodoacetamide or 5 mM-N-ethylmaleimide, and homogenized thoroughly in a tight fitting glass homogenizer, and stirred for 30 min on ice. The suspension was diluted to 0.5% Triton X-100 and centrifuged for 2 h at 100 000 g at 4°C. The Triton X-100 extract (30 ml) was mixed with 300 µl of choriogonadotropin-Affigel 10 and agitated overnight at 4°C end over end. The gel beads were collected by centrifugation at 250 g for 5 min and packed into a column (0.5 cm × 1.5 cm). The column was washed with 5 ml of phosphate-buffered saline containing 0.5% Triton X-100, followed by 5 ml of phosphate-buffered saline containing 0.1% Triton X-100 and 0.5 M-NaCl. These washes removed most of the contaminating proteins. The column was further washed with 2 ml of 10 mM-Tris/HCl, pH 7.0, containing 0.1% sodium deoxycholate, when an M_r 60 000 contaminant was washed off, and finally with 1 ml of phosphate-buffered saline containing 0.1% Triton X-100. Flow rate during the washes was 0.5 ml/min. The washed gel was eluted with 0.5 ml of 0.1 M-sodium acetate, pH 4.0, containing 0.1% Triton X-100, and the eluate was neutralized by 0.5 M-NaOH. For a control, the pseudopregnant rat ovaries were saturated with choriogonadotropin by injecting 500 units of the hormone into the tail vein 2 h before killing.

Assay of receptor activity

Particulate ovarian receptors were determined as described earlier (Rajaniemi *et al.*, 1977), and receptors solubilized by Triton X-100 were determined as described by Dufau *et al.* (1973). ¹²⁵I-choriogonadotropin for the determinations was prepared by the chloramine-T method of Hunter & Greenwood (1962) as described previously (Markkanen *et al.*, 1980).

SDS/polyacrylamide-gel electrophoresis

Samples were concentrated by evaporation at 40°C by an air stream and dialysed into a buffer

containing 2% (w/v) SDS, 125 mM-Tris/HCl, pH 6.8, 10% (v/v) glycerol, and 0.001% Bromophenol Blue. Electrophoresis was performed using the Laemmli (1970) buffer system. Gels were stained with Coomassie Blue (Fairbanks *et al.*, 1971), except for gels containing ^{125}I -radioactivity, which were cut into 1 mm slices and the slices counted for radioactivity with a gamma counter (LKB Wallac). Gels were calibrated with the molecular weight markers myosin (M_r 200 000), phosphorylase b (M_r 94 000), bovine serum albumin (M_r 66 000) and ovalbumin (M_r 45 000). The molecular weight markers were reduced by boiling for 10 min in the Laemmli sample buffer containing 1% 2-mercaptoethanol.

Reduction followed by alkylation was accomplished by boiling the sample for 10 min in a buffer containing 2% (w/v) SDS, 1% (v/v) 2-mercaptoethanol, 50 mM-Tris and 350 mM-glycine, pH 8.3, whereafter 1 M-iodoacetate was added (25 μl /100 μl sample), and the pH adjusted to 8.0. The solution was incubated for 10 min in the dark, whereafter 5 μl of 2-mercaptoethanol was added, and the solution was dialysed against the Laemmli sample buffer in the dark.

Extraction of the receptor polypeptide from an SDS/polyacrylamide-gel

The receptor polypeptide was located on the gel by using a Coomassie Blue-stained gel run in parallel. The polypeptide band was excised by a razor blade, and homogenized in a glass homogenizer in 5 vol. (500 μl) of 0.2% SDS. The homogenate was agitated overnight at 20°C, and centrifuged at 1000 g for 10 min. The extraction was repeated once, and the extracts were combined. A slice of a blank gel run in parallel without sample was processed identically, when no contaminating proteins were observed by Coomassie Blue staining.

Radioiodination of the receptor polypeptide

The receptor polypeptide band excised and extracted from an unstained gel was dialysed for 3 days against 50 mM-sodium phosphate, pH 7.0, containing 0.2% SDS. The nondiffusable material was evaporated into 50 μl , and radioiodinated with the chloramine-T method (Hunter & Greenwood, 1962). In detail, 0.5 mCi (5 μl) of Na^{125}I solution was mixed with the sample followed by 2 μg (10 μl) of chloramine-T three times at 1 min intervals. Free radioiodine was separated from the labelled product by gel filtration on a column of Sephadex G-25 (1.5 cm $\times$ 10 cm). The column was eluted with phosphate-buffered saline containing 0.2% SDS (0.5 ml/min), and 0.5 ml fractions were collected.

Gel filtration

Sephacryl S-300 was packed into a column

(1.5 cm $\times$ 80 cm) and equilibrated and eluted with phosphate-buffered saline containing 0.1% Triton X-100 at 4°C. Flow rate was 0.5 ml/min, and fractions (1.4 ml) were collected. The column was calibrated by Blue Dextran for void volume and by Na^{125}I for total volume.

Density gradient centrifugation

Centrifugation in 5–20% (w/v) sucrose gradients in phosphate-buffered saline containing 0.1% Triton X-100 was performed according to Martin & Ames (1961) in a Beckman model L5-50B ultracentrifuge, employing an SW-40 rotor. Radioiodinated samples of 100 μl were applied with marker proteins of known sedimentation coefficients: ^{125}I -choriogonadotropin (2.9 S), bovine serum albumin (4.3 S) and fibrinogen (7.9 S). After centrifugation for 20 h at 100 000 g at 4°C, fractions (250 μl) were collected, counted for radioactivity and protein was determined.

Protein determination

Protein was determined by the method of Lowry *et al.* (1951) as modified by Dulley & Grieve (1975).

Results

Glutaraldehyde crosslinking

Treatment with glutaraldehyde of the purified ^{125}I -choriogonadotropin–receptor complex did not affect its molecular size. The retardation coefficient of the complex on a column of Sephacryl S-300 remained unchanged ($K_{av} = 0.18$) when compared with the complex not treated with glutaraldehyde. In contrast to the native complex, the complex treated with glutaraldehyde was resistant to decomposition when incubated for 5 min in 100 mM-sodium acetate, pH 4.0, and decomposed only 30% when incubated for 1 h at 20°C in the presence of 4 M-guanidine hydrochloride containing 2 M-acetic acid, as was demonstrated by gel filtration. These observations show that glutaraldehyde essentially stabilizes the complex. The glutaraldehyde-treated ^{125}I -choriogonadotropin–receptor complex was subjected to electrophoresis on 7% polyacrylamide gels in the presence of SDS, when a major band with M_r 130 000 was observed in addition to those due to ^{125}I -choriogonadotropin (M_r 48 000) and its α -subunit (M_r 23 000) which carried the ^{125}I label (Markkanen *et al.*, 1980) (Fig. 1). For a control, pure ^{125}I -choriogonadotropin was attached to the antibody gel and displaced with an excess of choriogonadotropin, and treated with glutaraldehyde identically to the sample. Subsequent polyacrylamide-gel electrophoresis in SDS revealed only bands of ^{125}I -choriogonadotropin and its α -subunit, indicating that crosslinking between the choriogonadotropin molecules due to glutaraldehyde was

Fig. 1. SDS/polyacrylamide-gel electrophoresis of ^{125}I -choriogonadotropin-receptor complex treated with glutaraldehyde

A sample of ^{125}I -choriogonadotropin-receptor complex (40 000 c.p.m.) purified by anti-choriogonadotropin-Sepharose 4B was incubated at 20°C in 0.5 mM-glutaraldehyde for 4 h, dialysed, and subjected to electrophoresis in SDS on a 7% (w/v) polyacrylamide gel. The gel was sliced and the slices (each 1 mm) were counted for radioactivity (●). For a control, pure ^{125}I -choriogonadotropin was attached to and displaced from the anti-choriogonadotropin-Sepharose 4B and subjected to electrophoresis in SDS, when only ^{125}I -choriogonadotropin (^{125}I -hCG) and its α -subunit were observed (○). An identical curve was obtained when the purified ^{125}I -choriogonadotropin-receptor complex was subjected to electrophoresis in SDS without treatment with glutaraldehyde. M_r markers were myosin (200 000), phosphorylase b (94 000), and bovine serum albumin (66 000).

negligible. The M_r 130 000 complex was not detected if glutaraldehyde treatment of the ^{125}I -choriogonadotropin-receptor complex was omitted (Fig. 1). The M_r 130 000 species was not affected when boiled in the presence of SDS. When boiled in SDS in the presence of 2-mercaptoethanol, 80% of the radioactivity of the M_r 130 000 complex migrated coinciding with the α -subunit of choriogonadotropin.

Purification of the luteinizing hormone/choriogonadotropin receptor

Extraction of pseudopregnant rat ovarian par-

Fig. 2. Gel filtration on a Sephacryl S-300 column of the receptor- ^{125}I -choriogonadotropin complex formed between the purified receptor and the hormone

The receptor was purified by affinity chromatography in the presence of 5 mM-*N*-ethylmaleimide from 300 mg of pseudopregnant rat ovarian tissue as described in the Experimental section. Neutralized pH 4 eluate of choriogonadotropin-Affigel 10 (100 μl) was incubated for 16 h at 4°C with 50 μl of ^{125}I -choriogonadotropin (^{125}I -hCG) (30 000 c.p.m., 1 ng), and applied to a column of Sephacryl S-300. Two discrete peaks were resolved (●), corresponding to the positions of ^{125}I -choriogonadotropin-receptor complex formed *in vivo* and ^{125}I -choriogonadotropin. Incubation of the neutralized pH 4 eluate with ^{125}I -choriogonadotropin in the presence of an excess of unlabelled choriogonadotropin abolished the ^{125}I -choriogonadotropin-receptor complex (○).

ticles with 1% Triton X-100 resulted in 60% recovery of the luteinizing hormone/choriogonadotropin receptor into soluble form, as was measured by quantitative binding studies of the particulate and solubilized binding sites with ^{125}I -choriogonadotropin. The receptors solubilized by Triton X-100 disappeared from the Triton X-100 extract during an overnight agitation with choriogonadotropin-Affigel 10 beads, as was determined by the ^{125}I -choriogonadotropin binding assay. This suggests that they were bound to the affinity matrix. The yield of choriogonadotropin binding activity in the sodium acetate eluate after the affinity chromatography was 5% of that present in the Triton X-100 extract. The molecular size of the ^{125}I -choriogonadotropin-re-

Fig. 3. SDS/polyacrylamide-gel electrophoresis on 9% (w/v) gels of the solubilized ovarian membrane proteins before and after purification by affinity chromatography

Ovarian membrane particles were solubilized with Triton X-100 in the absence (lane a) and presence (lane b) of 10 mM-iodoacetamide. The extracts were subjected to affinity chromatography on choriogonadotropin-Affigel 10. Elution of the affinity gels with sodium acetate, pH 4.0, revealed an M_r 90 000 major polypeptide band regardless of whether iodoacetamide was absent (lane c) or present (lane d) during the affinity chromatography, but the presence of iodoacetamide enhanced the yield of the M_r 90 000 polypeptide. When ovaries saturated with choriogonadotropin were subjected to affinity chromatography in the presence of 10 mM-iodoacetamide, the M_r 90 000 band was abolished (lane e). For lanes (c)–(e), 1 g of ovarian tissue was used as starting material. Identical results were obtained in five separate experiments.

ceptor complex formed between ^{125}I -choriogonadotropin and the affinity-purified receptor was analysed by gel filtration on Sephacryl S-300. A complex with comparable size to that solubilized by Triton X-100 from pseudopregnant ovaries was observed (Fig. 2).

The quantity of protein recovered by affinity chromatography was $12\text{ }\mu\text{g/g}$ of ovarian tissue. This protein was subjected to polyacrylamide-gel electrophoresis in SDS on 9% gels, when an M_r 90 000 major band was observed (Fig. 3, lane c). Further elution of the affinity matrix with 4 M-guanidine hydrochloride containing 2 M-acetic acid showed no M_r 90 000 band on electrophoresis in SDS, suggesting that a quantitative recovery of this polypeptide was obtained by elution with sodium acetate at pH 4.0. Saturation of the receptors by choriogonadotropin before the purification procedure abolished the M_r 90 000 band. Use of iodoacetamide enhanced the yield of the M_r 90 000 polypeptide over twofold, as was determined by densitometric

Fig. 4. SDS/polyacrylamide-gel electrophoresis on 9% gels of the isolated M_r 90 000 luteinizing hormone receptor polypeptide before and after reduction

The receptor was purified by choriogonadotropin-Affigel 10 in the presence of 10 mM-iodoacetamide, subjected to SDS/polyacrylamide-gel electrophoresis and the M_r 90 000 band was excised and extracted. The extracted band moved homogeneously on electrophoresis in SDS (lane a), but boiling in 2% SDS containing 1% 2-mercaptoethanol followed by alkylation by iodoacetic acid revealed bands in the M_r 65 000 range in addition to the M_r 90 000 band (lane b). Identical results were obtained from three separate SDS/polyacrylamide-gel bands excised and extracted.

quantification of the Coomassie Blue-stained bands. Iodoacetamide caused appearance of additional M_r 45 000 and 46 000 bands (Fig. 3, lane d), which disappeared together with the M_r 90 000 band when tissue saturated with choriogonadotropin was used as starting material (Fig. 3, lane e). These bands were not present in the Triton X-100 extract without iodoacetamide, but they appeared when iodoacetamide was included (Fig. 3, lanes a and b) which suggests that they were just impurities.

The M_r 90 000 band was excised from an unstained polyacrylamide gel and extracted. Re-electrophoresis on 7% and 9% gels revealed a single band (Fig. 4). Densitometric scanning of the

Coomassie Blue-stained band showed that about 50% yield was obtained during the extraction procedure. The extracted band showed a tendency to aggregate when boiled in 2% (w/v) SDS in the presence of 1% (v/v) 2-mercaptoethanol, unless alkylated with iodoacetate. When reduced and alkylated, faster moving bands in the M_r range 60 000–65 000 were observed in addition to the M_r 90 000 band (Fig. 4). The M_r 90 000 band stained by the HIO_4 /Schiff method (Fairbanks *et al.*, 1971), indicating that it contained carbohydrate.

The ^{125}I -labelled receptor polypeptide

The M_r 90 000 band excised and extracted from a polyacrylamide gel was labelled with Na^{125}I in SDS medium. Successful labelling of the polypeptide was demonstrated by SDS gel electrophoresis, when an M_r 90 000 radioactive band was observed (Fig. 5).

Fig. 5. SDS/polyacrylamide-gel electrophoresis on a 9% (w/v) polyacrylamide gel of the radioiodinated M_r 90 000 receptor polypeptide

The luteinizing hormone receptor was purified by choriogonadotropin-Affigel 10 and subjected to polyacrylamide-gel electrophoresis. The M_r 90 000 band was excised from the gel and radioiodinated, and 150 000 c.p.m. applied to an SDS/polyacrylamide-gel. The radioiodinated product moved as an M_r 90 000 species as was determined by using standard proteins: myosin (M_r 200 000), phosphorylase b (M_r 94 000), albumin (M_r 66 000) and ovalbumin (M_r 45 000). Each slice was 1 mm.

This band was soaked from the gel, dialysed into phosphate saline containing 0.1% Triton X-100, and analysed further.

The ^{125}I -labelled polypeptide obtained by the above procedure recognized antibodies to purified luteinizing hormone receptor. When the ^{125}I -labelled polypeptide was incubated with an antiserum specific to the ovarian choriogonadotropin-binding sites (Metsikkö & Rajaniemi, 1981) in a 1:100 dilution, 42% of the radioactivity was precipitated with 10% (v/v) polyethylene glycol. Only 17% of radioactivity was precipitated in the control tubes where preimmune serum was used. The ^{125}I -labelled polypeptide bound quantitatively to concanavalin A coupled to Sepharose 4B, supporting the glycoprotein nature of the radioiodinated species. It could

Fig. 6. Sucrose density gradient centrifugation of the ^{125}I -labelled receptor polypeptide and ^{125}I -choriogonadotropin-receptor complex

The ^{125}I -labelled receptor polypeptide sedimented as a single band in a 5–20% (w/v) sucrose gradient (○) showing a sedimentation coefficient of $4.8 \pm 0.3 \text{ S}$ (s.d.) in five separate determinations (inset). The purified ^{125}I -choriogonadotropin-receptor complex (●) showed a sedimentation coefficient of $6.3 \pm 0.3 \text{ S}$ (s.d., five determinations; inset). hCG, choriogonadotropin; ^{125}I -R, ^{125}I -labelled receptor polypeptide; ^{125}I -hCG-R, ^{125}I -human choriogonadotropin-receptor complex.

not be displaced from the gel by 1 M- α -methyl mannoside. Gel filtration in the presence of 0.1% Triton X-100 on a column of Sephacryl S-300 showed a single peak with a retardation coefficient (K_{av}) of 0.25 ± 0.02 (s.d.), in five separate determinations. This was a significantly higher value than that of the ^{125}I -choriogonadotropin-receptor complex purified by immunoaffinity chromatography, for which a value of $K_{av} = 0.18 \pm 0.02$ (s.d.; five determinations) was obtained. An aliquot of the ^{125}I -labelled polypeptide was subjected to density gradient centrifugation in a 5–20% sucrose gradient containing 0.1% Triton X-100, when a sedimentation pattern showing a single peak was observed (Fig. 6).

Discussion

Significant progress has occurred recently regarding the purification of plasma membrane hormone receptors (Shiu & Friesen, 1974; Jacobs *et al.*, 1980; Heinrich *et al.*, 1980), but there is still lack of sufficient amounts of purified receptor material for further analysis. Consequently, covalent affinity labelling methods have been extensively employed to characterize the hormone binding components of plasma membrane receptors (Massague *et al.*, 1980; Yip *et al.*, 1980; Ji & Ji, 1981; Rebois *et al.*, 1981). Application of these methods to the luteinizing hormone receptor has not produced a uniform picture of the hormone binding component (Ji & Ji, 1981; Rebois *et al.*, 1981). Although the affinity labelling method is specific, its use has been limited to impure membrane systems. We have crosslinked purified ^{125}I -choriogonadotropin-receptor complex in a dilute solution utilizing glutaraldehyde as a crosslinker. Polyacrylamide-gel electrophoresis in SDS revealed an M_r 130 000 major polypeptide (Fig. 1), indicating that a single receptor component was linked to the hormone.

Purification of luteinizing hormone binding sites from pseudopregnant ovarian Triton X-100 extract revealed an M_r 90 000 major band on SDS/polyacrylamide-gel electrophoresis. This band was abolished when ovaries saturated by choriogonadotropin were used as starting material (Fig. 3), indicating that the M_r 90 000 species bound choriogonadotropin. It is reasonable to assume that the M_r 90 000 species is a component of the ^{125}I -choriogonadotropin-receptor complex, and was linked to ^{125}I -choriogonadotropin when the complex was treated with glutaraldehyde, producing the M_r 130 000 band observed by SDS/polyacrylamide-gel electrophoresis.

However, M_r values of about 200 000 have been derived for the detergent-solubilized receptor and its hormone complex (Dafau *et al.*, 1973; Dafau & Catt, 1976), and a ^{125}I -choriogonadotropin-receptor complex with comparable size was derived in

this study (Fig. 2). It is not known whether the M_r 200 000 receptor is a dimer of two M_r 90 000 units, whether the other half of it is detergent, or whether there is a non-hormone-binding unit or units associated with the M_r 90 000 unit. In the case of β -adrenergic receptor it has been shown that a guanine nucleotide binding protein is associated with the receptor in the presence of agonist (Limbird *et al.*, 1980). It is most probable that the luteinizing hormone receptor is composed of two M_r 90 000 components. It cannot be excluded, however, that the M_r 200 000 receptor contains a non-hormone-binding unit or units, which are washed off during the affinity chromatography.

The M_r 90 000 polypeptide we observed by Coomassie Blue staining after affinity chromatography probably represents the M_r 100 000 species that Rebois *et al.* (1981) have observed by the affinity crosslinking technique in rat testis. The present polypeptide had identical mobility with the receptor protein purified by immunoaffinity chromatography (Metsikkö & Rajaniemi, 1980) when compared on the same gel, although an M_r value of 110 000 has been derived using different standard proteins and a polyacrylamide gradient gel system. A choriogonadotropin complex with a molecular size about half that of the detergent-solubilized choriogonadotropin-receptor complex has been found to dissociate from pseudopregnant rat ovary during perfusion (Rajaniemi *et al.*, 1982). However, M_r 73 000, 76 000, 96 000, and 120 000 components have been observed after photoaffinity crosslinking of the radiolabelled α -subunit of choriogonadotropin to porcine granulosa cells (Ji & Ji, 1981). This finding cannot be reconciled with the above data. Also, a hormone binding component of M_r approx. 40 000 as determined by means of gel filtration has been shown to dissociate from rat Leydig cell membrane fraction (Pahnke & Leidenberger, 1978).

The isolated M_r 90 000 polypeptide moved homogeneously on 7% and 9% SDS/polyacrylamide gels, but showed faster-moving components when boiled with 2-mercaptoethanol and alkylated prior to electrophoresis (Fig. 4). The luteinizing hormone receptor protein purified by immunoaffinity chromatography totally turns into an M_r 65 000 species upon reduction, and in addition tends to aggregate (Metsikkö & Rajaniemi, 1980). The faster moving components possibly represent proteolytic fragmentation during the purification procedure of a polypeptide chain containing disulphide bonds. Proteolytic degradation produces variability in the subunit pattern of purified acetylcholine receptor (Vandlen *et al.*, 1979), and probably also occurs for the insulin receptor (Massague *et al.*, 1981). It remains to be determined whether the M_r 71 500 and 82 250 ^{125}I -choriogonadotropin complexes detected

by Pahnke & Leidenberger (1978) and the M_r 73 000, 76 000 and 96 000 ^{125}I -choriogonadotropin- α subunit complexes reported by Ji & Ji (1981) include the present M_r 60 000–65 000 fragments of the M_r 90 000 receptor unit.

The yield of active receptors in this study was only 5%, although a yield of over 100% has been reported applying affinity chromatography to rat testicular luteinizing hormone receptors (Dufau *et al.*, 1975). It is plausible that in this study the receptors denatured very rapidly at the low pH in a medium containing very little protein. However, it remains possible that a non-hormone-binding unit is required for high-affinity hormone binding, as has been shown for β -adrenergic receptor (Limbird *et al.*, 1980).

A major result of this study was the identification and purification of the luteinizing hormone binding polypeptide. The overall subunit composition of the M_r 200 000 receptor remains to be established.

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References

- Dufau, M. L. & Catt, K. J. (1976) in *Cell Membrane Receptors for Viruses, Antigens and Antibodies, Polypeptide Hormones and Small Molecules* (Beers, R. F. & Bassett, E. G., eds.), pp. 135–163, Raven Press, New York
- Dufau, M. L., Charreau, E. H. & Catt, K. J. (1973) *J. Biol. Chem.* **248**, 6973–6982
- Dufau, M. L., Charreau, E. H., Ryan, D. & Catt, K. J. (1974) *FEBS Lett.* **39**, 149–153
- Dufau, M. L., Ryan, D. W., Baukal, A. J. & Catt, K. J. (1975) *J. Biol. Chem.* **250**, 4822–4824
- Dulley, J. R. & Grieve, P. A. (1975) *Anal. Biochem.* **64**, 136–141
- Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
- Heinrich, J., Pilch, P. F. & Czech, M. P. (1980) *J. Biol. Chem.* **255**, 1732–1737
- Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496
- Jacobs, S., Hazum, E. & Cuatrecasas, P. (1980) *J. Biol. Chem.* **255**, 6937–6940
- Ji, I. & Ji, T. H. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7167–7170
- Ji, I. & Ji, T. H. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5465–5469
- Ji, I., Yoo, B. Y., Kaltenbach, C. & Ji, T. H. (1981) *J. Biol. Chem.* **256**, 10853–10858
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Limbird, L. E., Gill, M. D. & Lefkowitz, R. J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 775–779
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Markkanen, S., Töllikkö, K., Jääskeläinen, K. & Rajaniemi, H. (1980) *Horm. Res.* **12**, 32–45
- Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372–1379
- Massague, J., Pilch, P. F. & Czech, M. P. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7137–7141
- Massague, J., Pilch, P. F. & Czech, M. P. (1981) *J. Biol. Chem.* **256**, 3182–3190
- Metsikkö, K. & Rajaniemi, H. (1979) *FEBS Lett.* **106**, 193–196
- Metsikkö, K. & Rajaniemi, H. (1980) *Biochem. Biophys. Res. Commun.* **95**, 1730–1736
- Metsikkö, K. & Rajaniemi, H. (1981) *Endocrinology* **109**, 1399–1403
- Pahnke, V. G. & Leidenberger, F. A. (1978) *Endocrinology* **102**, 814–823
- Pandian, M. R. & Bahl, O. P. (1977) *Arch. Biochem. Biophys.* **182**, 420–436
- Parlow, A. F. (1961) in *Human Pituitary Gonadotropins* (Albert, A., ed.), pp. 300–310, C. C. Thomas, Springfield, IL
- Rajaniemi, H. J., Midgley, A. R., Duncan, J. A. & Reichert, L. E. (1977) *Endocrinology* **101**, 898–910
- Rajaniemi, H., Manninen, M., Metsikkö, K. & Huh-taniemi, I. (1982) *Biochim. Biophys. Acta* **714**, 271–278
- Rebois, R. V., Omedeo-Sale, F., Brady, R. O. & Fishman, P. H. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2086–2089
- Shiu, R. P. C. & Friesen, H. G. (1974) *J. Biol. Chem.* **249**, 7902–7911
- Vandlen, R. L., Wu, W. C.-S., Eisenach, J. C. & Raftery, M. A. (1979) *Biochemistry* **10**, 1845–1854
- Yip, C. C., Yeung, C. W. T. & Moule, M. L. (1980) *Biochemistry* **19**, 70–76