SOLUBILIZATION OF A PROSTAGLANDIN $F_{2\alpha}$ RECEPTOR IN BOVINE CORPORA LUTEA

Sven HAMMARSTRÖM, Ulf KYLDÉN, William S. POWELL and Bengt SAMUELSSON
Department of Chemistry, Karolinska Institutet, S-104 01 Stockholm 60, Sweden

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1. Introduction

The luteolytic effect of $PGF_{2\alpha}$* was first discovered in the rat [1]. It has later been demonstrated that this prostaglandin has a luteolytic effect in many other mammalian species too (for review see [2]). Furthermore, it has been shown that $PGF_{2\alpha}$ is a luteolytic hormone in the sheep [3]. It is released from the uterus and transported to the ovaries via a vascular pathway at the time of luteolysis.

We have recently demonstrated specific receptors for $PGF_{2\alpha}$ in ovine [4], bovine [5] and human [6] corpora lutea. These receptors were associated with particulate fractions from the corpora lutea [4–6] and could be inactivated by treatment with proteases, phospholipase A or *N*-ethylmaleimide [4]. This report describes the solubilization of the $PGF_{2\alpha}$ receptor from bovine corpora lutea.

2. Materials and methods

Bovine ovaries were collected and stored as previously described [5]. Detergents were obtained from the following sources: sodium dodecyl sulphate from Kebo AB, Stockholm, Sweden; sodium deoxycholate and Triton X-100 from Sigma Chemical Company; Nonidet P-40 from Shell Chemical Company. Sepharose 6B and Sephadex G-50 fine were products of Pharmacia Fine Chemicals, Uppsala, Sweden.

[9β-^{3}H]$PGF_{2\alpha}$ (1 Ci/mmol) and [17,18-$^3H_2$]-$PGF_{2\alpha}$ (22.5 Ci/mmol) were prepared as described before [7,8]. Unlabeled $PGF_{2\alpha}$ was generously supplied by Dr J. Pike of the Upjohn Company (Kalamazoo, Mich.). A Packard Tri Carb, model 3385, liquid scintillation spectrometer, equipped with automatic external standardization was used for radioactivity measurements. Protein was determined by the method of Lowry et al. [9] using bovine serum albumin as standard.

2.1. *Particulate fraction with $PGF_{2\alpha}$-binding activity*

The preparation of this fraction has been described before [5]. The material sedimenting between 1000 – 35000 *g* was resuspended in 0.01 M Tris–HCl buffer, pH 7.5, to give a protein concentration of 15 mg/ml.

2.2. *Incubations*

The resuspended particulate fraction was incubated at 23°C for 2 hr with [9β-^{3}H]$PGF_{2\alpha}$ or a mixture of [9β-^{3}H]$PGF_{2\alpha}$ and [17,18-$^3H_2$]$PGF_{2\alpha}$ (50 – 100 ng $PGF_{2\alpha}$ per ml of resuspended particulate fraction). The control for nonspecific binding contained in addition a 500-fold excess of unlabeled $PGF_{2\alpha}$. After incubation, the mixtures were kept on ice for 15 min before the addition of detergents.

2.3. *Solubilization*

Detergent solutions (1%, w/v) were prepared in 0.01 M Tris–HCl buffer, pH 7.5. The solutions were chilled to 0°C (12°C for sodium dodecyl sulphate) and one vol of detergent solution was added to the incubation mixtures for total as well as for non-specific binding. After 30 min at 0°C the mixtures were centrifuged at 270 000 *g* and 0°C for 1 hr in a Beckman, model L5-65, preparative ultracentrifuge. The clear supernatant, below a layer of lipid droplets, was removed by aspiration and assayed for soluble

* Prostaglandin $F_{2\alpha}$.

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$PGF_{2\alpha}$–receptor complex. Aliquots of 1 ml were frozen in a dry-ice ethanol mixture or chromatographed directly on columns of Sepharose 6B.

2.4. *Assay for $PGF_{2\alpha}$–receptor complex*

A previously described assay [4] based on the separation of bound and free ^{3}H-labeled $PGF_{2\alpha}$ by chromatography on 2-ml columns of Sephadex G-50 fine was used to assay both membrane bound and soluble $PGF_{2\alpha}$–receptor complex. The assays were carried out in duplicates or triplicates and the results were corrected for nonspecific binding.

2.5. *Sepharose chromatography*

Columns of Sepharose 6B were packed in Pharmacia manufactured water jacketed glass columns, model K 16/100, equipped with flow adaptors. The columns were equilibrated at 0°C for several weeks with detergent containing buffers as described in the legends to the figures. Fractions were collected in preweighed tubes and the volumes (0.7–0.9 ml) were measured gravimetrically. The void volume (V_0) and the total volume (V_t) of Sepharose columns were determined as the elution volumes (V_e) for blue dextran and [^{14}C]glucose, respectively. Elution volumes of other compounds were converted to K_{AV} values ($K_{AV} = \frac{V_e - V_0}{V_t - V_0}$, [10]).

3. Results

Table 1 shows the effect of four detergents on the binding reaction between $PGF_{2\alpha}$ and the corpus luteum receptor. At a concentration of 0.05% (w/v) the detergents inhibited the specific binding of $PGF_{2\alpha}$ by between 40 and 75% whereas at the same concentration of detergent the solubilization of protein was less than 5% above the control with buffer alone. However, 10-fold higher concentrations of Triton X-100, Nonidet P-40 and sodium deoxycholate had no adverse effects on the preformed $PGF_{2\alpha}$–receptor complex (fig.1, open bars) and assay for $PGF_{2\alpha}$–receptor complex in the 270 000 *g* supernatants showed that the complex was solubilized quantitatively by 0.5% (w/v) sodium deoxycholate and to an extent of 70–80% by 0.5% (w/v) Triton X-100 or Nonidet P-40 (fig.1, diagonal bars). Sodium dodecyl sulphate, 0.5% (w/v), dissociated the complex completely (fig.1), probably by denaturing the receptor. There was no solubilization of the $PGF_{2\alpha}$–receptor complex in a control incubation with 0.01 M Tris–HCl buffer alone.

Table 1
Effect of detergents on the binding reaction between $PGF_{2\alpha}$ and its receptor in bovine corpora lutea

Detergent (0.05% w/v)	Specific binding of $PGF_{2\alpha}$ (%)
None	5.20
Sodium dodecyl sulphate	1.35
Triton X-100	3.05
Nonidet P-40	3.15
Sodium deoxycholate	2.46

[9β-^{3}H]$PGF_{2\alpha}$, (100 ng), resuspended particulate fraction (15 mg of protein/ml, 0.95 ml) and detergent solution (1%, w/v 0.05 ml) or 0.01 M Tris–HCl, pH 7.5 (0.05 ml) were mixed and incubated at 23°C for 2 hr. Specific binding was assayed as described in the text and [4].

The effect of freezing and thawing $PGF_{2\alpha}$–

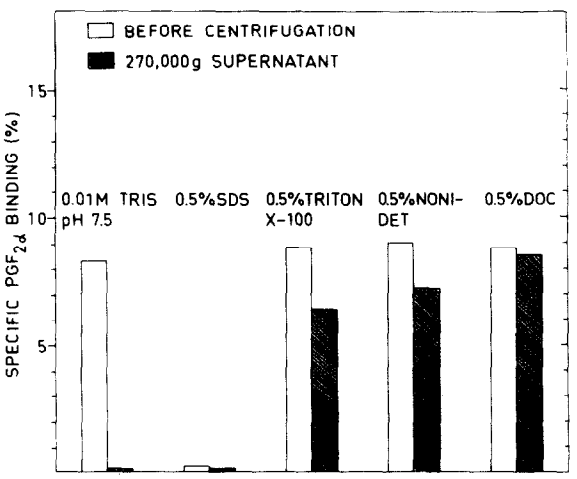

Fig.1. Effects of detergents on preformed [9β-^{3}H]$PGF_{2\alpha}$–receptor complex. A particulate fraction from bovine corpora lutea was incubated with [9β-^{3}H]$PGF_{2\alpha}$ to form $PGF_{2\alpha}$–receptor complex. After cooling to 0°C, 1% (w/v) detergent solutions were added to give final detergent and protein concentrations of 0.5% (w/v) and 7.5 mg/ml, respectively. After 30 min at 0°C, part of the mixtures were centrifuged at 270 000 *g* for 30 min. The amount of $PGF_{2\alpha}$–receptor complex was assayed in noncentrifuged samples (open bars) and in 270 000 *g* supernatant fractions (diagonal bars).

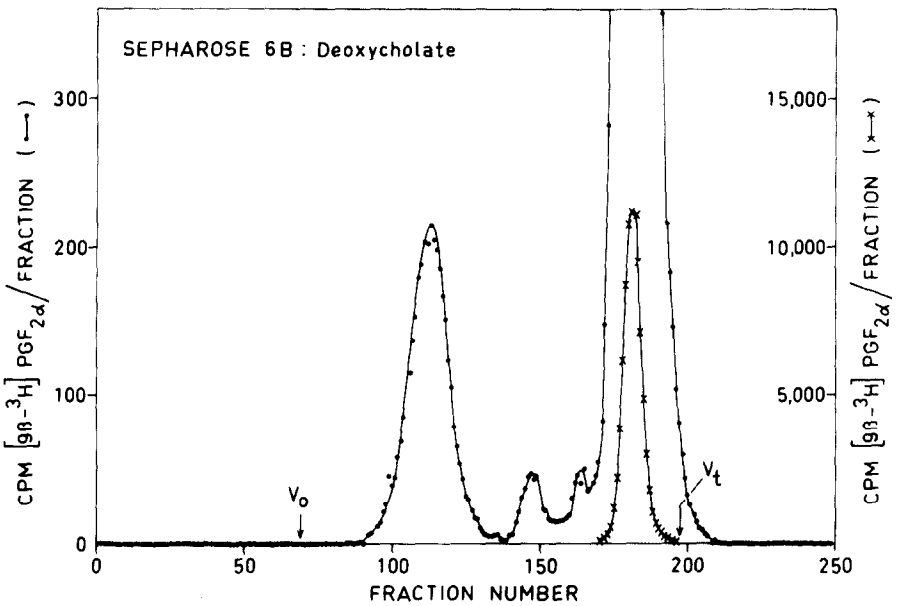

Fig.2. Chromatogram of sodium deoxycholate solubilized [9β-^{3}H]$PGF_{2\alpha}$–receptor complex on Sepharose 6B. [9β-^{3}H]$PGF_{2\alpha}$–receptor complex was formed and solubilized by treatment with sodium deoxycholate as described in the legend to fig.1 except that the centrifugation was for 2 hr at 270 000 *g*. A one ml aliquot of the supernatant was applied to a column of Sepharose 6B (1.6 × 84 cm) which had been equilibrated with 0.01 M Tris–HCl, pH 7.5–0.5% (w/v) sodium deoxycholate for four weeks. The flow rate was 4.7 ml/hr and the size of the fractions about 0.85 ml.

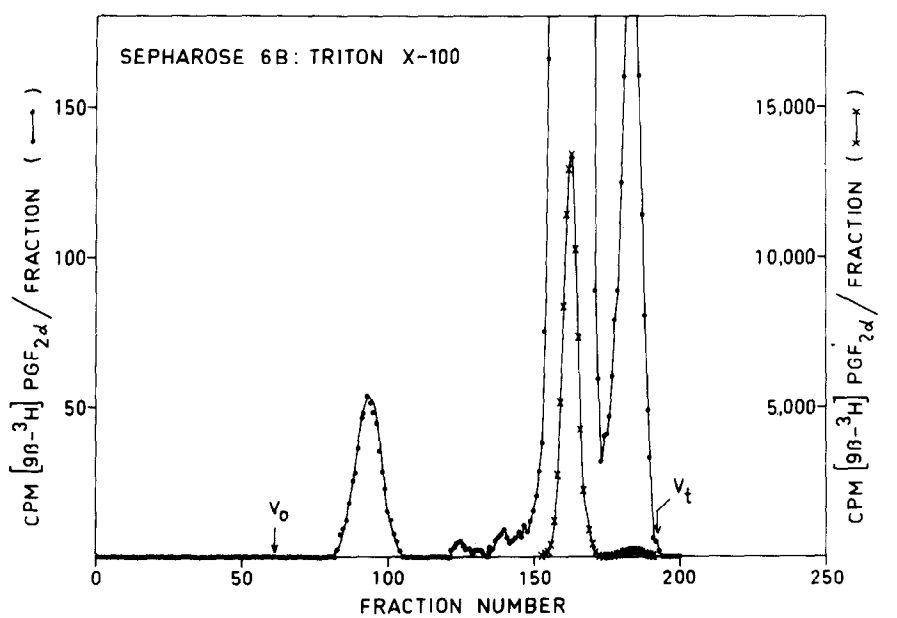

Fig.3. Chromatogram of Triton X-100 solubilized [9β-^{3}H]$PGF_{2\alpha}$–receptor complex on Sepharose 6B. The conditions for incubation and solubilization were those described in the legend to fig.2, with the exception that Triton X-100 was used instead of sodium deoxycholate. The column (1.6 × 81 cm) had been equilibrated with 0.01 M Tris–HCl, pH 7.5–0.5% (w/v) Triton X-100 for seven weeks. The flow rate was 4 ml/hr and the size of the fractions about 0.84 ml.

receptor complex solubilized by sodium deoxycholate was tested in one experiment: 98% and 93% of specifically bound $PGF_{2\alpha}$ remained after the first and fourth thawings, respectively. In another experiment, the dissociation of soluble $PGF_{2\alpha}$–receptor complex at 23°C, 0°C, –20°C and –80°C in the presence of 0.5% (w/v) sodium deoxycholate was followed versus time. After 2 hr at 23°C, about 67% of the complex remained, whereas at 0°C the same percentage of the complex remained after 168 hr. No measurable decrease in specifically bound $PGF_{2\alpha}$ took place at either –20°C or –80°C during a period of four weeks.

Fig.2 shows a chromatogram of sodium deoxycholate solubilized $PGF_{2\alpha}$–receptor complex on a column of Sepharose 6B which was eluted with Tris buffer containing 0.5% (w/v) sodium deoxycholate. The void volume (59 ml) and the total volume (168 ml) are indicated in fig.2. The two major radioactive components had elution volumes of 97 and 155 ml, respectively (K_{AV} values of 0.35 and 0.88). All of the soluble $PGF_{2\alpha}$–receptor complex which was applied to the column was recovered in the first peak. It corresponded to 3.8% of the total radioactivity. Furthermore, when sodium deoxycholate solubilized material from a control incubation for nonspecific binding (cf. [4]), was chromatographed on the same column, the first peak was absent showing that this peak represents the soluble [9β-^{3}H]$PGF_{2\alpha}$–receptor complex. The fourth peak had the same elution volume as free [9β-^{3}H]$PGF_{2\alpha}$, chromatographed under the same conditions. Chromatography of the solubilized $PGF_{2\alpha}$–receptor complex on Sepharose 6B in the absence of sodium deoxycholate changed the K_{AV} value for the $PGF_{2\alpha}$–receptor complex to 0, indicating that reaggregation of receptor molecules had occurred.

Fig.3 shows a chromatogram of Triton X-100 solubilized $PGF_{2\alpha}$–receptor complex on a column of Sepharose 6B using 0.01 M Tris, pH 7.5–0.5% (w/v) Triton X-100 as eluant. The void volume and the total volume were 51 and 161 ml, respectively, as indicated on the chromatogram. The first peak, representing the $PGF_{2\alpha}$–receptor complex, had an elution volume of 77 ml and a K_{AV} value of 0.24. The two other components, both representing free $PGF_{2\alpha}$, had elution volumes of 137 and 154 ml, respectively. When sodium deoxycholate solubilized $PGF_{2\alpha}$–receptor complex was chromatographed on Sepharose 6B in the presence of 0.5% (w/v) Triton X-100 the K_{AV} value of the $PGF_{2\alpha}$–receptor complex was 0.24, i.e., the same as for the Triton X-100 solubilized complex.

4. Discussion

Prostaglandin receptors have recently been demonstrated in several tissues (for review see [11]). The present paper is the first report on the solubilization of such a receptor. However, several other membrane bound hormone receptors have been reported to be solubilized by treatment with detergents, e.g., an insulin receptor in fat cells [12], β-adrenergic receptors in canine myometrium [13] and turkey erythrocytes [14], acetylcholine receptors in the electric organs of *Electrophorus electricus* and *Torpedo marmorata* [15], and a glucagon receptor in rat liver [16]. In this report, ^{3}H-labeled prostaglandin $F_{2\alpha}$ was preincubated with a particulate fraction from bovine corpora lutea to form the $PGF_{2\alpha}$–receptor complex. The complex rather than the free receptor, was rendered water soluble by treatment with one of the non-ionic detergents Triton X-100 or Nonidet P-40 or with the anionic detergent sodium deoxycholate. This procedure was used because the binding reaction between $PGF_{2\alpha}$ and the receptor was sensitive to detergents and since the receptor required detergent to be kept in solution. The radioactive prostaglandin served as a convenient label for the soluble $PGF_{2\alpha}$–receptor complex during chromatographies and centrifugations. Sodium deoxycholate and Triton X-100 solubilized $PGF_{2\alpha}$–receptor complexes were eluted within the fractionation range of Sepharose 6B when chromatographed in the presence of detergents. The Triton X-100 solubilized complex had a lower K_{AV} value than the sodium deoxycholate solubilized complex. Furthermore the latter complex had the same K_{AV} value as Triton X-100 solubilized complex when chromatographed in the presence of Triton X-100. This indicates that Triton X-100 was bound to the soluble receptor molecules. Studies of the interaction of deoxycholate and Triton X-100 with membrane proteins have recently been published [17,18].

In summary, the present results show that the

$PGF_{2\alpha}$–receptor in bovine corpora lutea can be solubilized by treatment with detergents. Purification and further characterization of the soluble hormone–receptor complex are in progress.

Acknowledgements

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