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PROSTAGLANDIN E AND $F_{2\alpha}$ RECEPTORS IN BOVINE CORPUS LUTEUM PLASMA MEMBRANES ARE TWO DIFFERENT MACROMOLECULAR ENTITIES

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SUMMARY

Greater numbers of prostaglandin (PG) $F_{2\alpha}$ as compared to PGE receptors were solubilized from bovine corpus luteum plasma membranes by sodium deoxycholate (SDC) concentrations from 0.01% to 0.1%. However, at 0.5% SDC concentration, virtually 100% of both PGs receptors were solubilized. When these solubilized PGs receptors were chromatographed on a calibrated Sepharose 6B column, they exhibited a small but reproducible difference in their distribution coefficients. The above results suggest that PGE and $PGF_{2\alpha}$ receptors represent two different macromolecular entities with a relatively small size difference between them.

INTRODUCTION

Recent studies have demonstrated the presence of prostaglandin (PG) E and $F_{2\alpha}$ receptors in intact viable cells (1), plasma membranes (2-6) and other subcellular fractions (6-9) prepared from bovine corpora lutea. The specificity and affinity of these receptors in cells (1) and plasma membranes (3,5) support the conclusion that PGE and $PGF_{2\alpha}$ receptors are discrete with respect to specificity and affinity. The use of various detergents and dimethylsulfoxide to probe the nature of membrane association of these receptors revealed that both PGE and $PGF_{2\alpha}$ receptors were intrinsic membrane proteins but the $PGF_{2\alpha}$ receptors were more peripheral with respect to PGE receptors in the membrane structure (10). The present study was undertaken to solubilize and to examine whether or not PGE and $PGF_{2\alpha}$ receptors are two different macromolecular entities.

MATERIALS AND METHODS

Unlabeled PGs were generously supplied by Dr. John Pike of the Upjohn Company. The following items were purchased from the indicated commercial sources: [^{3}H]PGE_1 (89.5 Ci/mmole) and [^{3}H]$PGF_{2\alpha}$ (178 Ci/mmole) from New

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England Nuclear Corp.; catalase (M.W.-232,000), ferritin (M.W.-400,000), thyroglobulin (M.W.-669,000) and Sepharose 6B from Sigma Chemical Co.; sodium deoxycholate (SDC) from Nutritional Biochemical Corp.; RPI Scintillator No. 111023 from Research Products International Corp.; Gelman Metricel filters (0.45 μm pore size) from Scientific Products and blue dextran 2000 from Pharmacia Fine Chemicals. Human hemoglobin (M.W.-64,500) was a generous gift from Dr. Robert Gray of our institution.

Both [^{3}H]PGs were checked for purity and if it was less than 95%, they were further purified as described earlier (10). Aliquots of [^{3}H]PGs stocks were diluted with redistilled ethanol and stored under nitrogen at -20^0 between uses. The procedures for the collection of bovine corpora lutea and isolation of purified plasma membrane fractions have already been described (6,11). These membranes exhibited about 32 fold enrichment of 5'-nucleotidase activity as compared to the homogenate (6). Plasma membrane fractions were stored in aliquots at -20^0 until used.

Aliquots of plasma membrane protein (3.2 to 14.0 mg in different experiments) were preincubated with either 30 nM [^{3}H]PGE$_1$ (38^0, 1 hr) or 10 nM [^{3}H] PGF$_{2\alpha}$ (22^0, 2 hrs), in 0.5 ml of 10 mM Tris-HCl, pH 7.3 containing 250 mM sucrose and 1 mM Ca^{2+}. Then 0.5 ml of 1% SDC in 10 mM Tris-HCl, pH 7.0 was added and incubation continued for another 1 hr at 4^0. Following this incubation, the tubes were centrifuged at 48,000 x g for 30 min at 4^0 and supernates were carefully decanted into clean tubes. Aliquots of the supernates were counted and the remainder was layered on a Sepharose 6B column (1.5 x 53 cm) and eluted with 10 mM Tris-HCl, pH 7.0 buffer containing 0.25% SDC (receptor aggregation occurred in SDC less than 0.25%) at 4^0 in fractions of 1.6 ml each. Absorbance at 280 nm and radioactivity in 0.5 ml aliquots of the fractions were measured. The radioactivity was measured (~20.0% counting efficiency) in a Packard liquid scintillation counter with 10 ml of scintillation fluid which consisted of toluene, Triton X-100, and RPI's scintillator No. 111023 in the ratio of 2750: 900:150 ml respectively. Protein was determined in selected column fractions (as well as in plasma membranes) according to Lowry et al (12) using bovine serum albumin as the standard. For column fractions, a blank containing detergent was employed.

The Sepharose 6B column was calibrated with hemoglobin, catalase, ferritin and thyroglobulin. These proteins were chromatographed using the same buffer as the solubilized PG receptors. The void volume (V_o) was determined as the elution volume for blue dextran 2000. The trailing end of the elution curve of [^{3}H]PGs was used to determine the total volume (V_t). K_{avs} (13) were calculated for the receptor bound [^{3}H]PGs and marker proteins. All the experiments were repeated at least three times. Other pertinent details are given in figure and table legends.

RESULTS

Table I shows that pretreatment of plasma membranes with increasing concentrations of SDC resulted in a progressively increasing solubilization of both PGE and PGF$_{2\alpha}$ receptors with virtually 100% solubilization occurring at 0.5% SDC. It should be noted, however, that a greater number of PGF$_{2\alpha}$ as compared to PGE receptors were solubilized at SDC concentrations from 0.01% to 0.1% with a maximal difference at 0.05% SDC.

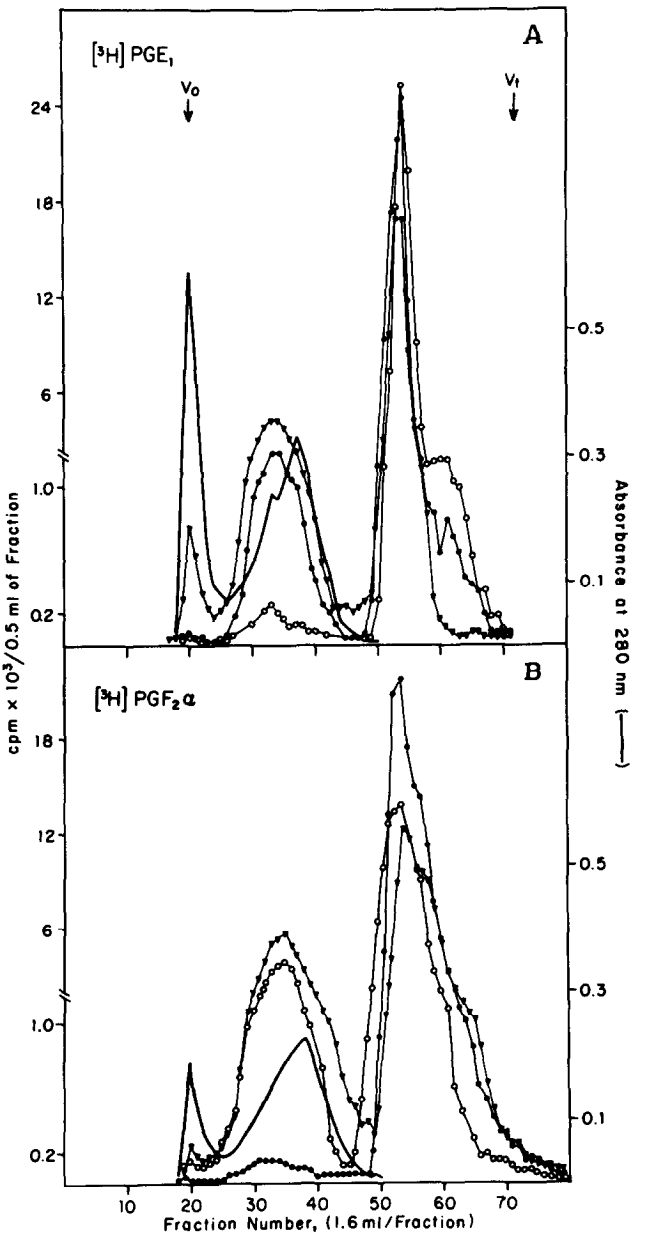
TABLE I

The Effect of Pretreatment of Plasma Membranes with Sodium Deoxycholate on Subsequent [^{3}H]PGs Specific Binding

Concentration of SDC (v/v), %	[^{3}H]PGE_1 Bound Percent of control	[^{3}H]$PGF_{2\alpha}$ Bound
0.01	97.0 ± 1.0	88.0 ± 2.0
0.05	68.0 ± 2.0	25.0 ± 1.0
0.1	14.0 ± 1.5	4.0 ± 1.7
0.5	0.0	0.0

Membrane protein aliquots were preincubated for 1 hr at 4^0 with increasing concentrations of SDC, centrifuged (6,000 x g, 15 min) and the pellets were washed once with 10 mM Tris-HCl, pH 7.0. The washed pellets were resuspended in 10 mM Tris-HCl pH 7.0 containing 250 mM sucrose, 1 mM Ca^{2+}, 1 mM dithiothreitol and 0.1% gelatin, aliquots of 200-600 µg protein were incubated with 12 nM [^{3}H]PGE_1 (38^0, 1 hr) and 6 nM [^{3}H]$PGF_{2\alpha}$ (22^0, 2 hrs). The rest of the details on this binding assay are the same as those described previously [9]. The membranes for controls were handled in a manner identical to treatment tubes except that SDC was not present during preincubation. The binding in control tubes, 69.0 and 143.1 fmoles/mg membrane protein for [^{3}H]PGE_1 and [^{3}H]$PGF_{2\alpha}$ respectively, was taken as 100%.

When SDC solubilized prelabeled PGE (Fig. 1A) and $PGF_{2\alpha}$ (Fig. 1B) receptors were chromatographed on a Sepharose 6B column, the protein was eluted in two peaks. The first came off the column with the void volume while the second protein peak corresponds to the first radioactive peak. The K_{av} for the first radioactive peak was 0.272 ± 0.014 for [^{3}H]PGE_1 and 0.289 ± 0.000 for [^{3}H]$PGF_{2\alpha}$. The rechromatography of the first radioactive peak following lypholization resulted in the same elution profile. The first radioactive peak represents macromolecular bound radioactivity, while the second radioactive peak represents free [^{3}H]PGs. The presence of excess unlabeled PGE_1 resulted in a complete inhibition of [^{3}H]PGE_1 binding (Fig. 1A) but it had very little effect on [^{3}H]$PGF_{2\alpha}$ binding (Fig. 1B) in their respective macromolecular peaks. Similarly, the presence of excess unlabeled $PGF_{2\alpha}$ resulted in a complete inhibition of [^{3}H]$PGF_{2\alpha}$ (Fig. 1B) but not of [^{3}H]PGE_1 binding (Fig. 1A) in their respective macromolecular peaks.



Figs. 1A & 1B - Gel filtration of free and [^{3}H]PGs-receptor complexes solubilized from bovine corpus luteum plasma membranes with SDC. Plasma membranes were preincubated with [^{3}H]PGs alone (closed triangles), or with [^{3}H]PGs and 5.6 x 10^{-6}M unlabeled PGE_1 (open circles) or 5.6 x 10^{-6}M unlabeled $PGF_{2\alpha}$ (closed circles) prior to solubilization. The average total recovery of the radioactivity from the column was 85.9% of which 26.8% was recovered in the macromolecular peak. Variation in fraction sizes (open and closed circles in 1B) required us to normalize the data before graphic display. The flow rate in this figure and in figure 2 was 1.6 ml/5 min.

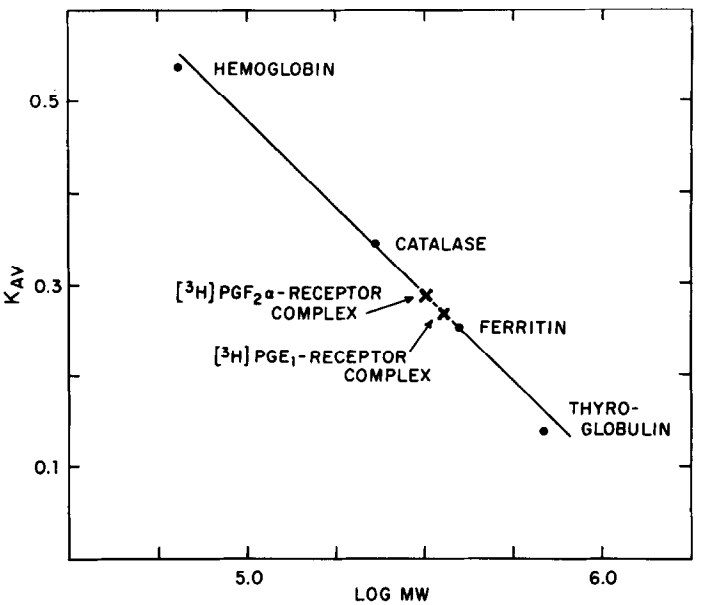


Fig. 2 - Estimation of molecular weights of solubilized PGE and $PGF_{2\alpha}$ receptor complexes by gel filtration on a calibrated Sepharose 6B column. The elution patterns for the marker proteins were followed by measurement of absorbances at 405 nm (hemoglobin, ferritin and thyroglobulin) and at 280 nm (catalase). The elution patterns for [^{3}H]PG-receptor complexes were followed by radioactivity measurements. The K_{avs} presented are the mean of 3 experiments.

In order to obtain a preliminary estimate of their molecular weights, a Sepharose 6B column used in the above studies was calibrated using four marker globular proteins whose molecular weights ranged from 64,500 to 669,000 (Fig. 2). The molecular weights of 346,736 and 323,953 for [^{3}H]PGE_1 and [^{3}H]$PGF_{2\alpha}$-receptor complexes respectively were obtained from the relationship between K_{av} vs log molecular weights of the marker proteins.

DISCUSSION

The SDC solubilized PGE and $PGF_{2\alpha}$-receptor complexes were non-sedimentable at g forces up to 322,000, not retained by Metricel filters of 0.45 μm pore size and not adsorbed by charcoal (data not shown). This indicated that SDC truly solubilized PGs-receptor complexes. Following gel filtration of SDC extracts on a Sepharose 6B column, the radioactivity appeared in two peaks, one of which corresponded to the macromolecular peak and the other to free [^{3}H]PGs.

The macromolecular radioactive peaks represent [^{3}H]PGs-receptor complexes but not [^{3}H]PGs-detergent micelle complexes because the binding in this peak exhibited a specificity expected of these receptors from studies with cells and plasma membranes (1-5).

Using a calibrated Sepharose 6B column, approximate molecular weights of 346,736 and 323,593 were assigned to the PGE-receptor and $PGF_{2\alpha}$-receptor complexes respectively, in the presence of SDC. Despite only 7.5% difference in molecular weights of PGE and $PGF_{2\alpha}$ complexes, the two receptors appear to represent two different macromolecular entities. If these two receptors were present on the same macromolecule, then these receptor losses due to solubilization from membranes should have been identical. But clearly they were not (see Table I).

The presence of detergent is quite essential for molecular weight determinations as its absence leads to inevitable receptor aggregation. Therefore, as long as detergent is present, problems such as possible anomalous SDC binding (14,15) to PG receptors and limitations involved in the comparison (13,16,17) of the filtration behavior of solubilized PGs receptors to that of marker proteins of known molecular weights are faced even in techniques that are more sophisticated (i.e. polyacrylamide gel electrophoresis) than simple gel filtration. This leaves one possible avenue for obtaining a molecular weight of the receptor corrected for detergent binding. This involves centrifugation of receptor complexes in sucrose density gradients containing H_2O and D_2O (18). Even in this procedure, certain assumptions have to be made. In view of the above discussion, the molecular weight estimates presented in this paper should be considered as preliminary and can serve as a basis for comparison with a wide variety of other membrane receptors molecular weights which were determined in the presence of detergents (reviewed in ref. 19).

There was one study which used an uncalibrated Sepharose 6B column to obtain a gel filtration pattern and a K_{av} (0.35) for $PGF_{2\alpha}$-receptor complexes solubilized from bovine corpus luteum plasma membranes (20). The difference

in K_{av} between our study and theirs (20) may perhaps be explained by different SDC concentrations used in the elution buffer.

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