THE PRESENCE OF DISCRETE RECEPTORS FOR PROSTAGLANDIN $F_2\alpha$ IN THE CELL MEMBRANES OF BOVINE CORPORA LUTEA

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SUMMARY

The cell membranes isolated from bovine corpora lutea bound ^{3}H-prostaglandin (PG) $F_2\alpha$ with high affinity and specificity. The specific binding of ^{3}H-$PGF_2\alpha$ was detectable at 10^{-10}M added ^{3}H-$PGF_2\alpha$ and reached saturation at 10^{-7}M to 10^{-6}M. Unlabeled $PGF_2\alpha$, as low as 10^{-9}M, inhibited the binding of ^{3}H-$PGF_2\alpha$ with complete inhibition occurring at 10^{-6}M. The Scatchard analysis of equilibrium binding data revealed that the $PGF_2\alpha$ receptors are heterogeneous: Kd_1-5.1 x 10^{-9}M, n-289 fmoles/mg protein; Kd_2-1.8 x 10^{-8}M, n-780 fmoles/mg protein. The relative affinities of various other PGs for binding to $PGF_2\alpha$ receptors were ($PGF_2\alpha$-100%): $PGF_1\alpha$-17.5; PGE_1-0.8; PGE_2-22.4; PGA_1-0.007; PGB_1-0.01. The specificity and affinity of ^{3}H-$PGF_2\alpha$ binding is consistent with the possibility that this receptor interaction may reflect an initial event in the action of $PGF_2\alpha$ as a luteolytic agent.

INTRODUCTION

Prostaglandin (PG) $F_2\alpha$ has been shown to be a potent luteolytic (demise of corpus luteum) substance in a number of animal species (reviewed in ref. 1). The physiological role of $PGF_2\alpha$ as a luteolytic agent has been well documented in a series of elegant experiments on sheep (2). Although the consequences of $PGF_2\alpha$ induced luteolysis are known (loss of gonadotropin receptors (3), decreased progesterone secretion (3) and structural changes (4)), the mechanisms involved that lead to these consequences are completely in the dark. With the advent of the availability of ^{3}H-$PGF_2\alpha$ of high specific activity, we have now been able to demonstrate the specific binding of $PGF_2\alpha$ to the cell membranes of bovine corpora lutea. The specificity and

affinity of $PGF_2\alpha$-receptor interaction is consistent with the suggestion that this receptor interaction may represent a biologically significant initial event in the action of $PGF_2\alpha$ as a luteolytic agent.

MATERIALS AND METHODS

Unlabeled PGs were generously donated by Dr. John Pike, the Upjohn Company, Kalamazoo, Mich. Unlabeled human chorionic gonadotropin (HCG) (10,600 IU/mg) was a gift from the Center for Population Research, N.I.C.H.H.D., N.I.H., Bethesda, Md. ^3H-$PGF_2\alpha$ (sp. act. 178 Ci/mmole) was purchased from New England Nuclear Corp. and checked for purity by thin layer chromatography using ethyl acetate: acetone: acetic acid (90:10:1 v/v) solvent system (5) and silica gel G sheets impregnated with 5% boric acid. If the purity was less than 98%, ^3H-$PGF_2\alpha$ was repurified by the above described thin layer chromatography (except 5% boric acid impregnation) prior to use in the binding studies. Aliquots of the ^3H-$PGF_2\alpha$ stock were diluted to 1 μCi/ml with redistilled ethanol and stored under nitrogen at -20° between uses.

The collection of bovine corpora lutea and the preparation of cell membrane fractions were the same as described before (6,7). The protein content in an aliquot of membrane fraction was determined by the method of Lowry et al (8) using bovine serum albumin as the standard.

^3H-$PGF_2\alpha$ (0.1 μCi) in redistilled ethanol was pipetted into 12 x 75 mm disposable glass tubes. The ethanol was blown dry under a stream of nitrogen. Aliquots of membrane fractions containing 360 μg protein were then added to the tubes and incubated for 1 hr at 22° (binding equilibrium was obtained at this time and temperature (9)). The final composition of incubation

buffer (final volume 0.2 ml) was 0.01 M Tris-HCl, pH 7.0, 0.25 M sucrose, 0.001 M $CaCl_2$, 0.001 M dithiothreitol and 0.1% gelatin. Following incubation, 1.0 ml of 0.01 M Tris-HCl buffer of pH 7.0 was added to each tube and poured onto Millipore filters (EHWP-0.5 μm pore size) positioned on a Millipore manifold under vacuum. The tubes were rinsed twice with 1.0 ml aliquots of 0.01 M Tris-HCl and filtered. Finally each filter was washed with 10.0 ml Tris-HCl buffer. The filters were then cut into halves and placed into scintillation vials containing 10.0 ml of scintillation fluid and counted in a Packard Scintillation counter with an average counting efficiency of 24.0%. The scintillation fluid consisted of toluene-Triton X-100-Packard Permafluor (25X) in the ratio of (%) 76:20:4 (v/v). Non-specific binding was assessed in each experiment by incubating aliquots of the membrane fractions with ^{3}H-$PGF_2\alpha$ and unlabeled $PGF_2\alpha$ (10^{-6}M). The non-specific binding was subtracted from total binding to obtain specific binding. The non-specific binding was essentially the same as the binding to the filters in the absence of membrane fractions (blanks) suggesting that non-specific binding was quite insignificant in these membrane fractions.

RESULTS

Fig. 1 shows that bovine corpus luteum cell membranes bound ^{3}H-$PGF_2\alpha$ at added ^{3}H-$PGF_2\alpha$ concentrations as low as 3.1×10^{-10}M, that binding increased with increasing amounts of ^{3}H-$PGF_2\alpha$ added, and finally reached saturation at 10^{-7} to 10^{-6}M.

The data in Fig. 1 was used in a Scatchard Plot analysis (10) (Fig. 2). It can be seen from this figure that $PGF_2\alpha$ binding was heterogeneous, indicating the presence of high affinity low capacity and low affinity - high capacity $PGF_2\alpha$ binding sites. There was about 3 fold difference in the affinities

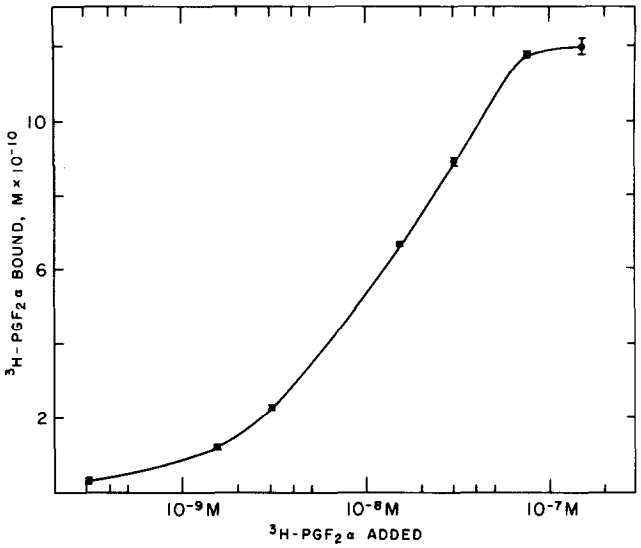

Fig. 1 Dependency of ^{3}H-PGF$_2\alpha$ specific binding to bovine corpus luteum cell membranes on the amounts of ^{3}H-PGF$_2\alpha$ added. Each observation in Figs. 1 and 3 represents the mean with its standard error.

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and the number of sites between these two groups of receptors.

The specificity of ^{3}H-PGF$_2\alpha$ binding is shown in Fig. 3. The addition of increasing amounts of unlabeled PGF$_2\alpha$ resulted in a dose dependent inhibition of ^{3}H-PGF$_2\alpha$ binding with complete inhibition occurring at 10^{-6}M. Unlabeled PGF$_1\alpha$ and PGE$_2$ were less effective and PGE$_1$ was least effective when compared to unlabeled PGF$_2\alpha$ in inhibiting ^{3}H-PGF$_2\alpha$ binding to the membranes.

The affinities of other PGs for binding to PGF$_2\alpha$ receptors were calculated from the concentrations of PGs needed for half maximal inhibition of ^{3}H-PGF$_2\alpha$ binding (Ki) to the membranes. The affinity of PGF$_2\alpha$ was taken as 100% and the relative percent affinities for other PGs and HCG were calculated and presented in Table I. As shown in this table, PGF$_1\alpha$ and PGE$_2$ had moderate affinities, whereas PGE$_1$, which binds to its own receptors in the same membranes, had 125 fold less affinity for PGF$_2\alpha$ receptors.

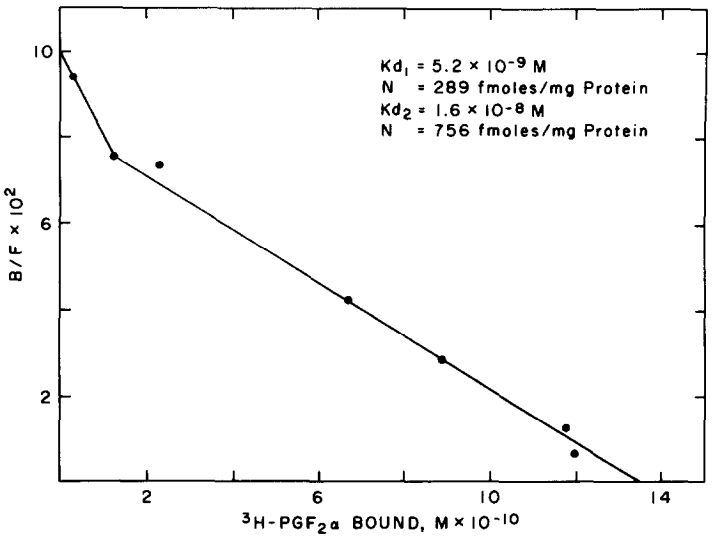

Fig. 2 The Scatchard Plot analysis of ^{3}H-$PGF_{2}\alpha$ binding data presented in Fig. 1.

PGA_1 and PGB_1 had 10,000 (or less) fold lower affinities and HCG, which binds to its own receptors in the same membranes, had no affinity at all for $PGF_{2}\alpha$ receptors.

DISCUSSION

^{3}H-$PGF_{2}\alpha$ bound to the outer cell membranes of bovine corpora lutea with high affinity and specificity. The Scatchard analysis of equilibrium binding data revealed the presence of two sets of receptors with a difference of about 3 fold with respect to affinities and number of sites. The affinity of ^{3}H-$PGF_{2}\alpha$-receptor interaction is in excellent agreement with the PGF levels (4.2 to 8.5 x 10^{-9}M) in uterine vein blood of cow (11) and the specificity parallels the potencies of PGs as luteolytic agents (12-15). Therefore, it is quite reasonable to suggest that $PGF_{2}\alpha$ binding may reflect physiologically significant receptor interaction. Although the consequences of $PGF_{2}\alpha$ induced luteolysis are known,

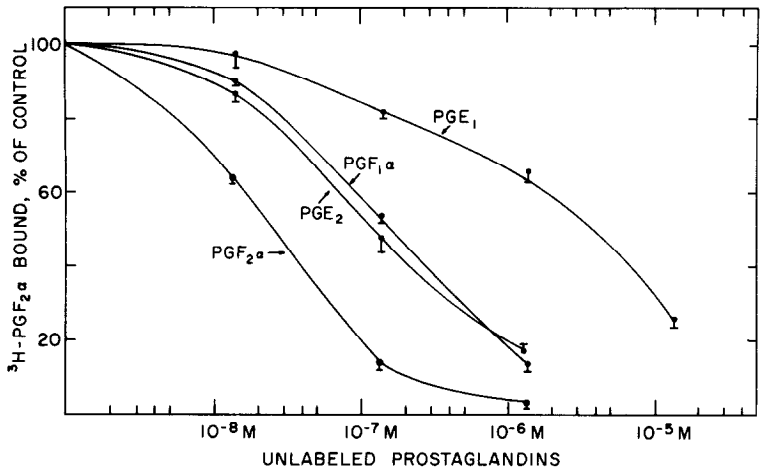

Fig. 3 The specificity of ^{3}H-PGF$_2\alpha$ binding to bovine corpus luteum cell membranes. Unlabeled PGs in ethanol (except PGF$_2\alpha$) were added and dried under nitrogen before the addition of membranes. Since PGF$_2\alpha$ was in the form of THAM salt, it was dissolved in 0.01 M Tris-HCl pH 7.0 buffer and added. The amount of ^{3}H-PGF$_2\alpha$ bound in the control tubes (contain no unlabeled PGs) was 94.7 fmoles/mg membrane protein which was taken as 100%.

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the mechanisms which lead to these consequences are completely in the dark. However, the presence of specific cell membrane receptors for PGF$_2\alpha$ may suggest the possibility that PGF$_2\alpha$-receptor interaction at the cell surface constitutes an initial event in a complex series of events in PGF$_2\alpha$ induced luteolysis.

Powell et al (16) have reported earlier on the presence of PGF$_2\alpha$ receptors in ovine corpora lutea. The following observations of these workers: homogenity of PGF$_2\alpha$ receptors, apparent dissociation constant (1 x 10^{-7}M) and relative affinity for PGF$_1\alpha$ (20 fold less as compared to only 5.7 fold less observed in this study) were not in agreement with the findings reported in this paper. However, some of these differences may perhaps be due to the differences between species and to the use of ^{3}H-PGF$_2\alpha$ of different specific activities (1 Ci/mmole vs 178 Ci/mmole used

Relative Affinities of Various Compounds for Binding to $PGF_2\alpha$ Receptors in Bovine Corpus Luteum Cell Membranes

Compound	Relative Affinity (Percent)
$PGF_2\alpha$	100.000
$PGF_1\alpha$	17.500
PGE_2	22.400
PGE_1	0.800
PGB_1	0.010
PGA_1	0.007
HCG	0.000

in our studies). It may be of interest to point out that Powell et al (16) have used for binding studies the membrane fragments obtained by centrifuging the 1000 x g supernate of the homogenate at 100,000 x g for 60 min. The affinity of $PGF_2\alpha$-receptor interaction reported by these workers is lower than the circulating levels of $PGF_2\alpha$ (8.5×10^{-9} to 6.5×10^{-8}M) in utero-ovarian vein blood of sheep (2).

Bovine corpus luteum cell membranes have been shown to be a locus of receptors for PGEs (17,18) and HCG (19). These receptors are different from $PGF_2\alpha$ receptors not only by virtue of their specificities but also by their other properties (9,20). Although PGEs and HCG binding to their receptors can be related to the activation of adenylate cyclase (21,22), $PGF_2\alpha$ binding may not be related to this enzyme activity as it has very little effect on the activity of this enzyme (21). Kuehl (23)

speculated that $PGF_2\alpha$ stimulates guanylate cyclase activity in ovaries in view of the observations that this compound is capable of increasing cyclic GMP levels in vascular tissues (24). Therefore, it may be possible that $PGF_2\alpha$-receptor interaction may be related to guanylate cyclase activity. Such a possibility is presently being investigated in our laboratory.

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