[^{3}H] PROSTAGLANDINS BINDING TO DISPERSED BOVINE LUTEAL CELLS: EVIDENCE FOR DISCRETE PROSTAGLANDIN RECEPTORS

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SUMMARY

Suspensions of dispersed bovine luteal cells prepared by collagenase digestion of luteal tissue specifically bound [^{3}H]Prostaglandin (PG) E_1 and [^{3}H]$PGF_{2\alpha}$. While the number of sites per cell ($\sim 1.8 \times 10^5$) were about the same for both [^{3}H]PGs, the apparent Kds were different: [^{3}H]PGE_1 - 2.4 nM; [^{3}H]$PGF_{2\alpha}$ - 11 nM. The [^{3}H]PGs binding was inhibited in a dose-dependent manner in the presence of increasing concentrations of unlabeled PGs. Potency order for inhibition of [^{3}H]PGE_1 binding was: $PGE_2 > PGE_1 > PGF_{2\alpha} > PGF_{1\alpha}$ The corresponding data for [^{3}H]$PGF_{2\alpha}$ was: $PGF_{2\alpha} > PGF_{1\alpha} > PGE_2 > PGE_1$. While [^{3}H]$PGE_1$ and [^{3}H]$PGF_{2\alpha}$ bind to their own receptors with high affinity, their affinities for each other's binding were extremely low. Thus, these results demonstrate that luteal cells, like plasma membranes isolated from luteal tissue, contain receptors for PGEs and $PGF_{2\alpha}$ which are discrete with respect to specificity and affinity.

INTRODUCTION

The purified and crude plasma membrane fractions of bovine corpora lutea have been shown to contain discrete prostaglandin (PG) receptors (1-3). One of these receptors binds PGEs (PGE_1 & PGE_2) and the other binds $PGF_{2\alpha}$ with high affinity and specificity (1-3). The binding of these PGs to two different receptor sites can be justified on functional as well as on minor structural differences grounds (PGEs contain a keto group on carbon 9 whereas PGFs contain a hydroxy group) (3). Since isolated plasma membranes do not come close to physiological situation, a documentation should be provided that discrete PG receptors do exist under conditions that are close to physiological situation, for example, in intact viable luteal cells. Therefore, we undertook the present studies and the results show that dispersed bovine luteal cells, like plasma membranes isolated from luteal tissue, do bind [^{3}H]PGE_1 and [^{3}H]$PGF_{2\alpha}$ with a specificity and affinity consistent with discreteness of these receptors.

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MATERIALS AND METHODS

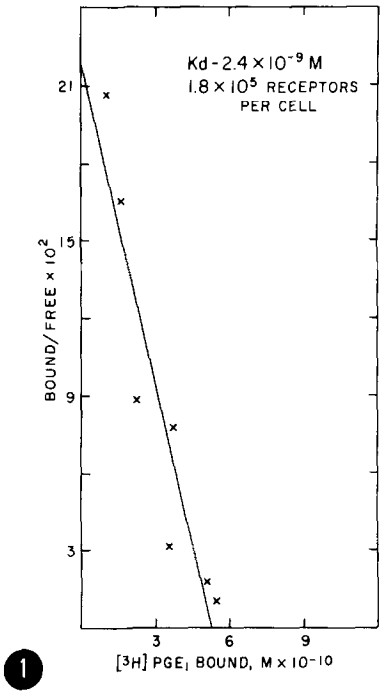
Bovine corpora lutea throughout pregnancy were used in these studies because they have been shown earlier to contain PGE and $PGF_{2\alpha}$ receptors (4, 5). Immediately following the collection in a local slaughterhouse, the tissues were placed in Krebs-Ringer phosphate buffer containing 0.2% glucose (KRPG) and transported on ice to the laboratory. The relatively homogeneous population of luteal cell suspensions were prepared by collagenase (Type 1 from Cl. histolyticum from Worthington Biochemicals) digestion of luteal tissue slices essentially by the method described by Simmons et al (6). The final cell pellet was suspended in Krebs-Ringer phosphate buffer containing 0.2% glucose and 1% bovine serum albumin (BSA) (KRPG-BSA buffer). Cell viability was assessed by a dye exclusion method (7) using 0.2% eosin. Cells excluding the dye were considered viable (7). The cell number was determined by counting in a haemocytometer.

Once the cells were prepared, they were used for up to 3 days because the binding was not significantly altered during this time period. Between uses, the cells were stored in Medium 199 (obtained from Grand Island Biological Co.) and KRPG-BSA (1:1) at 4°. Each day before their use, the cell viability and number were determined. Cell membranes appeared to be intact when examined under a light microscope (x430). The yield of cells per gm of luteal tissue varied from 3 to 9 x 10^7 in different experiments. The proportion of big cells to small cells varied from batch to batch and the cell number used per experiment was based on total cells. The luteal cells were functional as judged by the production of substantial amounts of progesterone.

[^{3}H]PGE_1 (89.5 Ci/mmole) and [^{3}H]$PGF_{2\alpha}$ (178 Ci/mmole) were purchased from New England Nuclear Corporation and checked for purity. If their purity was less than 95%, they were further purified as described earlier (8). The details on [^{3}H]PGs binding to luteal cells were the same as those described earlier for plasma membranes (8-10) except for the following: the buffer composition during incubation was the same as KRPG-BSA and incubation times and temperatures were 90 min at 38° for [^{3}H]PGE_1 and 3 hrs at 22° for [^{3}H]$PGF_{2\alpha}$. In the initial experiments, Gelman Metricel filters (obtained from Scientific Products) of 0.45 μm and 1.2 μm pore sizes were compared in trapping the cell bound radioactivity and no significant differences were found. Therefore, 0.45 μm pore size filters were subsequently used in all the experiments. The non-specific binding was determined in each experiment using the same cell number and [^{3}H]PGs concentrations but in the presence of 2.8 x 10^{-5}M corresponding unlabeled PGs (generously donated by Dr. J. Pike of the Upjohn Company). The specific binding, which is presented in all the tables and figures, was the difference between total and non-specific binding. The non-specific binding constituted about 20% of the total binding and was essentially the same as the binding to the filters in the absence of cells (blanks) suggesting that it was quite insignificant. The molar concentration of [^{3}H]PGs bound were calculated using the molecular weight of 354 for both PGs. All the experiments were run in quadruplicates and repeated at least twice. Although there was variability in cells with respect to binding from experiment to experiment, various binding parameters, for example, constants and specificity were almost identical. Because of the above reason, the data of all the experiments were not pooled. Thus, each experimental value was the mean of four observations in one such representative experiment. The rest of the experimental details are given in table and figure legends.

RESULTS

The Scatchard analysis (11) of [^{3}H]PGE_1 binding to the luteal cells reveals that there was a homogeneous population of receptors with an apparent



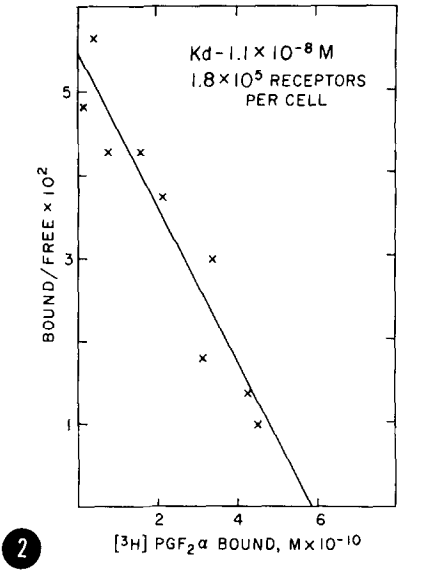


Fig. 1 Scatchard analysis of [^{3}H] PGE_1 binding to luteal cells. Aliquots of 1.8 x 10^5 cells were incubated with increasing concentrations of [^{3}H] PGE_1 (1 nM to 56.5 nM). The resulting specific binding data was transformed and graphically displayed according to Scatchard (11). The slope and the intercept were determined by linear regression analysis. The reciprocal of the slope gave a Kd value and multiplication of the x intercept value with Avagadro's number and division by cell number per liter gave the number of receptor sites per cell.

Fig. 2 Scatchard analysis of [^{3}H] $PGF_2\alpha$ binding to luteal cells. Aliquots of 2 x 10^5 cells were incubated with increasing concentrations of [^{3}H] $PGF_2\alpha$ (1 nM to 52 nM). The rest of the details were the same as in Figure 1 legend.

dissociation constant (Kd) of 2.4 nM and an apparent number of receptor sites of 1.8 x 10^5 per cell (Fig. 1).

Figure 2 shows that the [^{3}H]$PGF_2\alpha$ binding to the luteal cells was also homogeneous with about the same number of receptors sites per cell as for [^{3}H]PGE_1. The Kd of 11 nM for [^{3}H]$PGF_2\alpha$ binding was however, different from that of [^{3}H]PGE_1. On occasions, we have observed a second low affinity site for both [^{3}H]PGE_1 and [^{3}H]$PGF_2\alpha$, the Kds of which could not be calculated because the lines had little if any measurable slope.

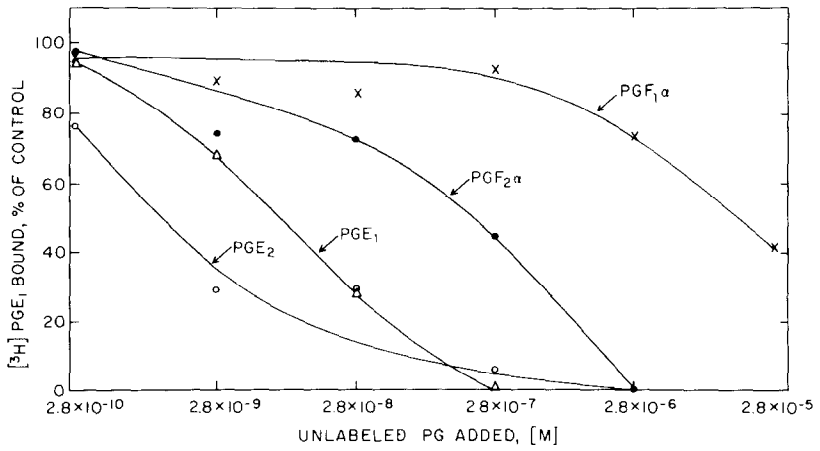


Fig. 3 Specificity of [^{3}H] PGE_1 binding to luteal cells. Aliquots of 1.8 x 10^5 cells were incubated with 10.6 nM [^{3}H] PGE_1 and increasing concentrations of unlabeled PGs. The binding in control tubes containing no unlabeled PGs was taken as 100%. All the unlabeled PGs were added in ethanol to the tubes containing [^{3}H] PGE_1 in ethanol and dried under nitrogen before the addition of cells.

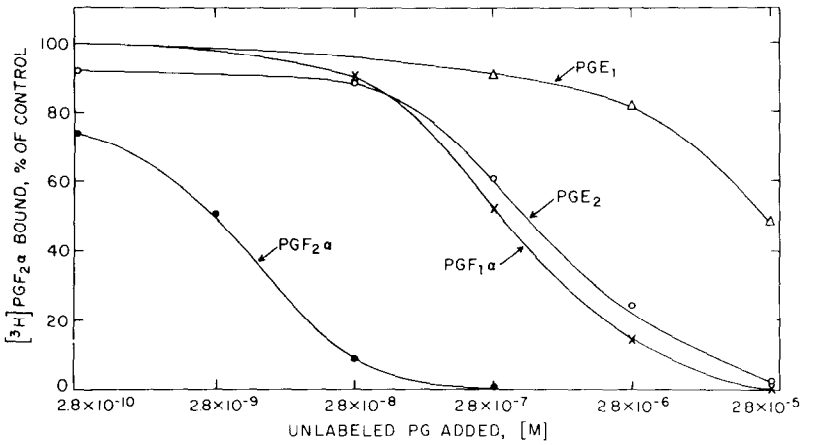


Fig. 4 Specificity of [^{3}H] $PGF_2\alpha$ binding to luteal cells. Aliquots of 1.8 x 10^5 cells were incubated with 6 nM [^{3}H] $PGF_2\alpha$ and increasing concentrations of unlabeled PGs. The rest of the details are the same as in Figure 3 legend.

The specificity of [^{3}H]PGE_1 binding to the cells is shown in Fig. 3. The presence of increasing amounts of unlabeled PGs inhibited [^{3}H]PGE_1 binding in a dose-dependent manner. The potency order for this binding inhibition was: $PGE_2 > PGE_1 > PGF_2\alpha > PGF_1\alpha$.

Fig. 4 presents the specificity of [^{3}H]$PGF_2\alpha$ binding to luteal cells. Once

TABLE 1

Relative affinities of various PGs for binding to PGE_1 and $PGF_{2\alpha}$ receptors in luteal cells.
The % relative binding affinities were calculated from the concentrations of PGs needed for half maximal inhibition of [^{3}H] PGs binding.

Unlabeled PG	[^{3}H] PGE_1	[^{3}H] $PGF_{2\alpha}$
	(Relative affinity %)	
PGE_1	100	0.01
PGE_2	718.2	0.6
$PGF_{1\alpha}$	0.05	0.9
$PGF_{2\alpha}$	4.2	100

again the [^{3}H]$PGF_{2\alpha}$ binding was inhibited in a dose-dependent manner in the presence of increasing amounts of unlabeled PGs. The potency order for this inhibition of binding was: $PGF_{2\alpha} > PGF_{1\alpha} > PGE_2 > PGE_1$.

Although [^{3}H]PGE_1 and [^{3}H]$PGF_{2\alpha}$ bind to luteal cells with high affinity, the discreteness of their binding is striking as can be seen in Fig. 3 & 4 and in Table 1. The relative affinities in Table 1 reveal that while PGE_1 and $PGF_{2\alpha}$ bind to their corresponding receptors with high affinity, they had very low affinity for each other's binding.

DISCUSSION

Plasma membranes isolated from appropriate target tissues have been widely used in a variety of radio ligand binding studies (Reviewed in ref. 12). In most of these studies, inferences drawn using plasma membranes have not been validated using a situation that is closer to being physiological, for example intact viable cells. This is important because various physical and other forces involved in tissue or cell disruption and subsequent fractionation could be suspected to alter receptor properties.

Previous reports from our laboratory as well as others demonstrated the presence of discrete PG receptors in bovine corpus luteum plasma membranes (1-3). One of these receptors bound [^{3}H]PGE_1 and the other bound [^{3}H]$PGF_{2\alpha}$ with high affinity and specificity. Although the presence of the above discrete PG receptors can be justified on functional as well as on minor structural differences grounds (3), it is desirable to demonstrate the presence of such discrete PG receptors in intact viable luteal cells which more closely approach physiological situation.

The results presented in this paper demonstrate that relatively homogeneous populations of luteal cells bound [^{3}H]PGE_1 and [^{3}H]$PGF_2\alpha$. Although the number of receptors were about the same for both ligands, the apparent Kds were different. The discreteness of these ligands binding is strikingly clear from the specificity data. While PGE_1 and $PGF_{2\alpha}$ bind to their own receptors with high affinity, they have remarkably low affinity for binding to each other's receptors. Thus the luteal cells, like plasma membranes isolated from luteal tissue, contain discrete PG receptors. The following differences were observed between cells and plasma membranes: a) both [^{3}H]PGs binding was monophasic in cells and biphasic in plasma membranes and, b) unlabeled PGE_2 was much more effective than PGE_1 in competition studies with cells. In plasma membranes, unlabeled PGE_1 and PGE_2 were about equally effective. c) The PG relative affinities for binding to PGE_1 and $PGF_{2\alpha}$ receptors in cells were generally lower than those observed in plasma membranes (3). The reasons for these differences between cells and plasma membranes can only be speculative at the present time.

ACKNOWLEDGEMENT

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