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STRUCTURAL REQUIREMENTS FOR PROSTAGLANDIN ANALOG INTERACTION WITH THE OVINE CORPUS LUTEUM PROSTAGLANDIN $F_{2\alpha}$ RECEPTOR

IMPLICATIONS FOR DEVELOPMENT OF A PHOTOAFFINITY PROBE

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Abstract—The capacity of structurally modified analogs of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to inhibit binding of [^{3}H]$PGF_{2\alpha}$ to receptors on ovine luteal cells was evaluated by radioreceptorassay using dispersed, viable, ovine luteal cells. Binding assays were conducted at pH 5.75, since binding to both high (K_d 17.4 ± 2.3 nM) and low (K_d 409 ± 166 nM) affinity sites was enhanced markedly at reduced pH. The capability to compete with [^{3}H]$PGF_{2\alpha}$ for binding was evaluated for different prostaglandin analogs having modifications in the C-8 "upper" side-chain, in the cyclopentane ring, or in the C-12 "lower" side-chain. Prostaglandin J_2 was a surprisingly potent competitor for binding to the $PGF_{2\alpha}$ receptor. Several phenyl-substituted analogs exhibited receptor-binding potency greater than or equal to native $PGF_{2\alpha}$, while most other analogs had reduced capacity to compete with native $PGF_{2\alpha}$ for binding. Several 17-azidophenol $PGF_{2\alpha}$ analogs were synthesized and tested, but analogs having hydroxyl groups on the aryl ring had low affinity for receptors. However, 17-(4-azidophenyl)-18,19,20-trinor-$PGF_{2\alpha}$ as well as 17-(3-iodo-4-azidophenyl)-18,19,20-trinor-$PGF_{2\alpha}$ exhibited binding affinities that were approximately 10% of native $PGF_{2\alpha}$, and the radioiodinated analogs of $PGF_{2\alpha}$ may be useful as probes of the $PGF_{2\alpha}$ receptor.

The mammalian corpus luteum produces progesterone which is essential for the successful establishment and maintenance of pregnancy. Consequently, manipulations of luteal function and lifespan may have profound influences upon reproduction. During approximately the past two decades, it has been demonstrated in many mammalian species that administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) *in vivo* causes luteal regression [1]. Luteal regression following $PGF_{2\alpha}$ administration in the ewe is manifested by a precipitous decline in circulating progesterone that is detectable within 7.5 hr [2], morphological changes in luteal cells within 12 hr [3], diminution of available receptors for luteinizing hormone within 22.5 hr [2], and tissue degeneration within 24–48 hr [3].

Despite rapid luteolysis following $PGF_{2\alpha}$ administration *in vivo*, $PGF_{2\alpha}$ has modest effects upon luteal function *in vitro*. Using the criterion of progesterone secretion by preparations of luteal tissue from various species, different investigators have characterized the effect of $PGF_{2\alpha}$ *in vitro* as slightly stimulatory [4–6], inhibitory [7], or without effect [8]. The puzzling lack of luteolytic potency of $PGF_{2\alpha}$ *in vitro* is especially interesting since corpora lutea have abundant receptors for $PGF_{2\alpha}$ [8]. Several biochemical responses of luteal tissue of $PGF_{2\alpha}$ treatment *in vitro* have been reported, including attenuation of luteinizing hormone-stimulated steroidogenic activity [9, 10], decreased membrane fluidity [11], and increased turnover of phosphatidylinositol [12–14]. However, none of these changes have been correlated convincingly with the luteolytic mechanism of $PGF_{2\alpha}$.

To examine the molecular mechanism of $PGF_{2\alpha}$ effects upon luteal cells and to attempt to resolve the disparity in $PGF_{2\alpha}$ actions *in vivo* and *in vitro*, we elected to develop photoaffinity analogs of $PGF_{2\alpha}$ for use as probes of receptor structure and function. The ovine corpus luteum was selected as a model tissue since the corpus luteum from this species has a well defined population of $PGF_{2\alpha}$ receptors as well as *in vivo* [15] responsiveness to $PGF_{2\alpha}$. To develop photoaffinity probes, azidoaryl substitutions of $PGF_{2\alpha}$ were examined since similar photoactive derivatives were successfully incorporated in photoaffinity probes for a variety of small as well as large ligands [reviewed in Ref. 16].

Utilization of intact, viable ovine luteal cells afforded the opportunity to examine not only receptor structure but also cellular responses to $PGF_{2\alpha}$. However, as we began development of $PGF_{2\alpha}$ photoprobes, it soon became apparent that specific binding of [^{3}H]$PGF_{2\alpha}$ to both high and low affinity sites was enhanced by incubations at acidic pH. Virtually

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all prior examinations by other investigators of $PGF_{2\alpha}$ binding to luteal tissue were conducted at physiological pH [reviewed in Ref. 17]. Reasoning that the microenvironment of the corpus luteum might become an acidic milieu during the early stages of luteal regression, we elected to conduct binding assays at pH 5.75. The relative binding affinities for binding to the high affinity sites were evaluated for a variety of analogs, metabolites, and other related eicosanoids, including several compounds that have not heretofore been examined in a $PGF_{2\alpha}$ receptor assay system.

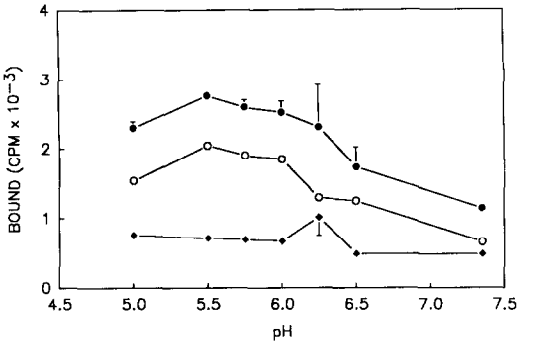

Fig. 1. Effect of pH on [^{3}H]$PGF_{2\alpha}$ binding to luteal cells. Aliquots containing 50,000 large luteal cells were incubated with 50 nCi [^{3}H]$PGF_{2\alpha}$ in 210 μl buffer in which pH was adjusted with NaOH or HCl. Incubations were conducted at 30° for 45 min. Bound radioactivity was quantified following filtration of incubates as described in Materials and Methods. Specific binding (○) was the calculated difference between total binding (●) and nonspecific binding in parallel incubations containing 1 μg nonradioactive $PGF_{2\alpha}$ (◆). Each data point represents a mean (± SD) of triplicate determinations.

MATERIALS AND METHODS

Reagents. Follicle stimulating hormone (FSH) was obtained from Burns Biotech (Omaha, NE), cronolone pessaries from Intervet International N.V. (Boxmeer, The Netherlands), and collagenase type IV from Cooper Biomedical (Malvern, PA). Deoxyribonuclease I, Hanks' buffered salt solution (HBSS) and Ca^{2+}/Mg^{2+}-free HBSS were from Whittaker MA Bioproducts (Walkersville, MD), bovine serum albumin Fraction V (BSA), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), Tris and dimethyl sulfoxide (DMSO) from Sigma Chemical Co. (St Louis, MO) and Whatman GF/B glass-fiber filters from Fisher Scientific (Richmond, VA). Handifluor scintillation fluid was from Mallinckrodt (Paris, KY). [^{3}H]$PGF_{2\alpha}$ (195 Ci/mmol) from New England Nuclear (Boston, MA), and LIGAND from Elsevier-Biosoft (Cambridge, UK). Eicosanoids containing either azido-modifications or C-1 methyl esters were synthesized by us [18]. Other eicosanoids were purchased from the Cayman Chemical Co. (Ann Arbor, MI).

Superovulation regimen. Estrus in ewes was detected using vasectomized rams. Progestogen pessaries were administered vaginally to ewes that were 8–12 days postestrus and removed after 10 days. Ewes received intramuscular injections of FSH twice daily beginning 72 hr prior to, and ending at the time of, pessary removal (5 mg first injection, 2.5 mg remaining injections). Most ewes exhibited estrus on the day following pessary removal. This regimen typically indiced 6–18 corpora lutea per animal, which were removed 8–10 days post-ovulation.

Corpora lutea dispersion. Ovariectomies and luteal dissociations were conducted using aseptic procedures similar to those described earlier [19], except that cells were harvested and combined from two 45-min incubations instead of a single 90-min incubation. Viability of dispersed cells was assessed using trypan blue exclusion [20].

Cryopreservation. Dispersed luteal cells were cooled to 4°, and DMSO was added to 7.5% (v/v) final concentration. Aliquots containing 10^6 large (18–35 μm diameter) luteal cells were distributed among cryotubes and frozen using a Cryo-Med model 801 programmable cell freezer (having linear cooling gradients from 4° to −38° in 42 min, then from −38° to −90° in 5 min). Frozen cells were transferred to liquid nitrogen and stored at −196°. Before use in radioreceptorassays, frozen cells were thawed in a water bath at 37°. Thawed cells were placed on ice, pelleted by centrifugation, washed and resuspended in assay buffer. Viability of thawed luteal cells was typically 60–90% as monitored by trypan blue exclusion. No decrease in viability of frozen cells was detectable after nitrogen storage for periods of up to 6 months.

Analog binding assays. Phenylazide eicosanoid analogs were dissolved in DMSO to concentrations of 10 mg/ml. Other eicosanoids were dissolved in ethanol to concentrations of 10 mg/ml. In preliminary experiments, neither solvent influenced binding of [^{3}H]$PGF_{2\alpha}$ at their maximum added concentrations (1 μl/210 μl total assay volume). Procedures involving azido-labeled analogs were conducted using minimal light. Aliquots of dispersed luteal cells containing 50,000 large luteal cells/tube, 50 nCi [^{3}H]$PGF_{2\alpha}$ (195 Ci/mmol), and competing eicosanoid as indicated were incubated in 210 μl total volume of assay buffer (HBSS–BSA adjusted to pH 5.75). Incubations proceeded in a gently shaking water bath for 45 min at 30°. Incubations were terminated by addition of 1.5 ml of ice-cold buffer (25 mM Tris, 1 mM $CaCl_2$, 0.1% BSA, pH 7.35); incubates were applied to glass microfiber filters on a Yeda filtration manifold, and were washed twice using 1.5-ml volumes of cold Tris–Ca–BSA buffer. Filters containing washed cells were placed in vials containing 10 ml of Handifluor scintillation fluid and equilibrated for 16 hr at 4°. Radioactivity was quantified using a Tracor Mark III liquid scintillation counter having a tritium detection efficiency of 44%. To correct for slight variations in nonspecific binding between different lots of cells, binding data were standardized by expression of analog displacement curves on a specific binding basis.

Displacement curves were constructed for all analogs tested, except for a few having minimal binding potency in which only several high concentrations of analog were tested. Using LIGAND [21], the binding affinity estimate was obtained for each compound tested, based on the displacement of native $PGF_{2\alpha}$

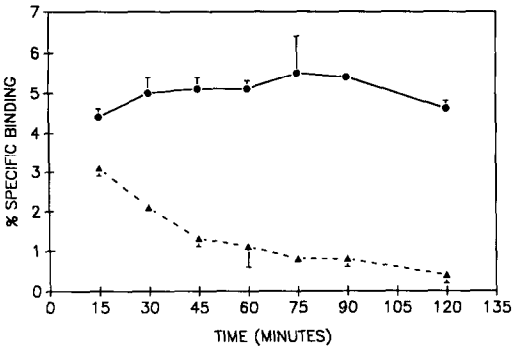

Fig. 2. Effects of time and temperature on specific binding of $PGF_{2\alpha}$ to luteal cells. Aliquots containing 50,000 large luteal cells and 50 nCi [^{3}H]$PGF_{2\alpha}$ in 210 μl assay buffer were incubated at 30° (●) or 37° (▲) for the indicated times. Incubations were terminated by filtration as described in Materials and Methods. Specific binding was the calculated difference between binding in the absence and presence of 1 μg $PGF_{2\alpha}$. Each data point represents a mean (± SD) of triplicate determinations.

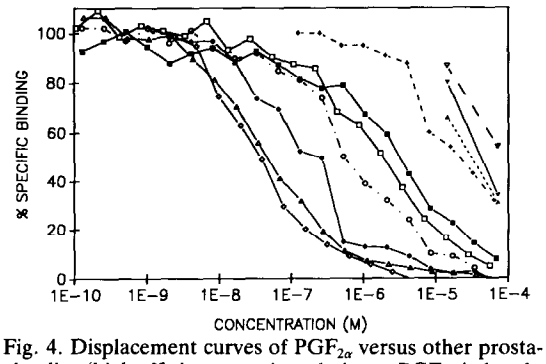

Fig. 4. Displacement curves of $PGF_{2\alpha}$ versus other prostaglandins (high affinity potencies relative to $PGF_{2\alpha}$ in brackets; see Materials and Methods for details). Key: (◇) $PGF_{2\alpha}$ [100%]; (△) PGJ_2 [36.6 ± 3.3%]; (●) PGD_2 [11.6 ± 2.4%]; (○) PGE_2 [1.9 ± 0.3%]; (□) 9β-$PGF_{2\alpha}$ [1.4 ± 0.1%]; (■) PGE_1 [0.5 ± 0.05%]; (◆) PGI_2 [0.3 ± 0.3%]; (▲) PGB_2 [< 0.1%]; (▼) PGB_1 [< 0.1%]; and (▽) PGA_2 [< 0.1%]. IE-10 = 10^{-10}.

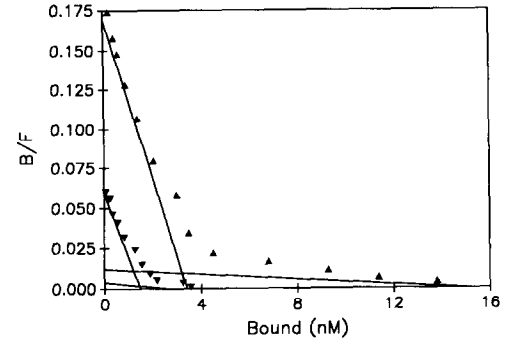

Fig. 3. Effect of pH on Scatchard analyses. Aliquots containing 50,000 large luteal cells and 50 nCi [^{3}H]$PGF_{2\alpha}$ and dilutions of native $PGF_{2\alpha}$ from 0 to 3 μM in 210 μl assay buffer [(▲) pH 5.75; (▼) pH 7.35] were incubated for 45 min at 30°. Incubations were terminated by filtration as described in Materials and Methods. Concentrations of bound radioligand were transformed to Scatchard plots using LIGAND.

from the high affinity $PGF_{2\alpha}$ site. The relative affinity then is the ratio of the affinity of the analog to the affinity of $PGF_{2\alpha}$.

RESULTS

Radioreceptorassay validation. The $PGF_{2\alpha}$ radioreceptorassay used for these studies was a departure from the usual regimen followed by most previous investigators in this field, who incubated membrane preparations at physiological pH. Specific binding of [^{3}H]$PGF_{2\alpha}$ was maximal at acidic pH and was attenuated markedly at physiological pH (Fig. 1). At pH 5.75, binding of [^{3}H]$PGF_{2\alpha}$ was maximal at incubations for 45 min at 30° (Fig. 2). Scatchard transformations of binding data revealed increased abundance of both high and low affinity binding at pH 5.75 compared with pH 7.35 (Fig. 3).

Displacement curves of [^{3}H]$PGF_{2\alpha}$ and relative high affinity potencies relative to $PGF_{2\alpha}$ for a variety of eicosanoids are depicted in Figs. 4–9. Results for other species of prostaglandins are depicted in Fig. 4; results for compounds having the cyclopentane ring of $PGF_{2\alpha}$ but with lower side-chain modifications are depicted in Fig. 5; results for compounds having multiple structural differences from $PGF_{2\alpha}$ are depicted in Fig. 6; results for analogs having lower side-chain simple phenyl substitutions are depicted in Fig. 7; results for analogs having lower side-chain azidophenol substitutions are depicted in Fig. 8; and results for analogs having lower side-chain azidophenyl substitutions are depicted in Fig. 9. Relative binding potencies (mean ± SD) of each analog relative to $PGF_{2\alpha}$ are included in the figure legends.

DISCUSSION

Dispersed cellular preparations of ovine corpora lutea retained $PGF_{2\alpha}$ binding properties. In preliminary experiments it was determined that frozen then thawed cells had $PGF_{2\alpha}$ binding properties that were not different from those of freshly dissociated cells. Furthermore, frozen then thawed cells retained viability that was similar to that of unfrozen cells. Therefore, the use of frozen ovine luteal cells relieved dependence upon the seasonal availability of ovine corpora lutea and may be useful for studies of cellular responsiveness to $PGF_{2\alpha}$.

We tested the capability of a variety of eicosanoids and $PGF_{2\alpha}$ analogs to bind to the luteal $PGF_{2\alpha}$ receptor. To clarify data presentation, analog displacement curves were grouped into categories (other prostaglandin species, lower side-chain modifications, miscellaneous modifications, aryl analogs, azidophenol analogs, and azidophenyl analogs) which were individually contrasted with native $PGF_{2\alpha}$. Error bars were not included to maximize clarity. For each displacement curve, data were connected point-to-point instead of using curve fitting

or linearization procedures so that data could be readily inspected for subtleties such as multicomponent displacement curves. The displacement curves were a composite of binding to both high and low affinity sites. However, utilization of LIGAND enabled the individual estimates of binding affinities to the high affinity site. Displacement curves for most compounds tested appeared to be parallel with the displacement curve for $PGF_{2\alpha}$, suggesting that the compounds were competitive inhibitors.

Our assignments of binding potency for different analogs of $PGF_{2\alpha}$ are not intended to reflect biological potency. In previous investigations, Kimball *et al.* [22] concluded that a high correlation did not always exist between $PGF_{2\alpha}$ analog receptor binding affinity and luteolytic potency and suggested that the disparities between binding and biological potencies may be due to resistance of some analogs to metabolic inactivation. Similar lack of correlation was also noted in other biological versus binding assays [23]. However, while binding and biological potencies of $PGF_{2\alpha}$ analogs are not always highly correlated, examples of marked disparity between biological and binding potencies are difficult to find.

The potent luteolytic effect of $PGF_{2\alpha}$ administered *in vivo* contrasts with its modest and variable effects *in vitro*. It was interesting that, in our system, the binding of $PGF_{2\alpha}$ was maximal at acidic pH (Fig. 1). Furthermore, Scatchard analyses revealed an increased number of both high and low affinity binding sites at pH 5.75 compared with pH 7.35 (Fig. 3). In one of the first demonstrations of a luteal receptor for $PGF_{2\alpha}$, Powell *et al.* [24] suggested that binding of $PGF_{2\alpha}$ to luteal receptors was greater at acid pH than in physiological pH. However, because of aggregation at acidic pH which interfered with separation of bound and free ligand, incubations were conducted at physiological pH. Subsequently, competitions of $PGF_{2\alpha}$ and analogs for binding to luteal receptors were almost universally examined in buffering systems having pH 7.0–7.5 [23–28]. Incubations conducted at pH 5.75 may cause cellular aggregation, which was not monitored in our studies. However, while aggregation compromised chromatographic separation of bound and free ligand in earlier studies [24], aggregation would not be expected to compromise the filtration separation procedure used in our studies. The maintenance of minimal nonspecific binding during incubations at pH 5.75 (Fig. 1) is suggestive that cell aggregation was not disruptive in our system. Additionally, while pH 5.75 is close to the pK_a of the carboxyl termini of prostaglandins, low levels of nonspecific binding were indicative that nonspecific, hydrophobic insertion of $[^3H]PGF_{2\alpha}$ into the cellular lipid bilayer was not a significant complication. The range of reported binding affinities for $PGF_{2\alpha}$ binding to luteal receptors was reviewed recently [17]. Previous investigators have variously reported a single affinity binding component having dissociation constants that were summarized in Ref. 17 as 27.4 ± 33.5 nM as well as multicomponent binding with a lower affinity component having a K_d of 113 ± 162 nM. These compare with the high (K_d 17.4 ± 2.3 nM) and low (K_d 409 ± 166 nM) affinities in our system.

Our utilization of intact, viable cells enabled estimation of receptor concentrations on a per cell basis. We standardized our $PGF_{2\alpha}$ radioreceptorassay on the basis of 50,000 large luteal cells per tube, although this cell number also includes other cells such as the "small" steroidogenic cells as well as nonsteroidogenic luteal cells. Using fractionation by elutriation, binding of $[^3H]PGF_{2\alpha}$ to ovine luteal cells was previously localized primarily to the population of "large" cells having diameters of 20–35 μm, and little binding was detected to other luteal cell subpopulations [19]. On the assumption that $PGF_{2\alpha}$ binding is indeed restricted to the large luteal cells, inspection of Scatchard analyses (Fig. 3) revealed an abundance of $PGF_{2\alpha}$ receptors that we estimate at > 2 million high affinity binding sites per large luteal cell.

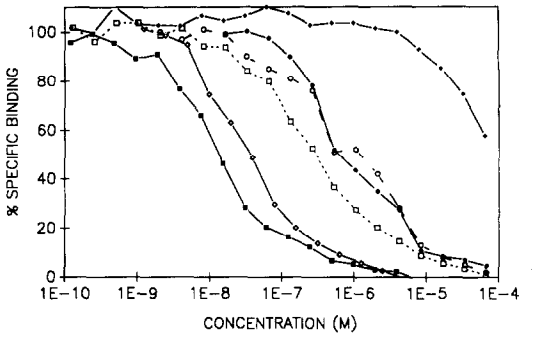

Fig. 5. Displacement curves of $PGF_{2\alpha}$ versus lower side-chain modifications (high affinity potencies relative to $PGF_{2\alpha}$ in brackets; see Materials and Methods for details). Key: (■) 16,16-dimethyl-$PGF_{2\alpha}$ [159.6 ± 63.5%]; (◇) $PGF_{2\alpha}$ [100%]; (□) (*R*)15-$PGF_{2\alpha}$ [6.7 ± 0.7%]; (●) 13,14-dihydro-15-keto-$PGF_{2\alpha}$ [1.4 ± 0.3%]; (○) 15-keto-$PGF_{2\alpha}$ [1.2 ± 0.5%]; and (◆) (*R*)19-hydroxyl-$PGF_{2\alpha}$ [0.02 ± 0.002%].

$PGF_{2\alpha}$–luteal interactions *in vitro* at physiological pH may not reflect the luteal milieu following $PGF_{2\alpha}$ administration *in vivo*. In studies of $PGF_{2\alpha}$ effects upon ovine luteal cells *in vitro* [7], it was noted that progesterone secretion was depressed by $PGF_{2\alpha}$ treatments in which medium was replenished at 6-hr intervals with fresh $PGF_{2\alpha}$-containing medium, but not when medium was replaced at hourly intervals. It was speculated that unknown "factors" might accumulate in medium in response to $PGF_{2\alpha}$ which exerted effects only after reaching critical levels. In view of our present observations at reduced pH, we would suggest an alternative mechanism for the reported $PGF_{2\alpha}$ effects only after infrequent medium changes. Infrequent medium changes may result in accumulation of acidic metabolic products that enhance binding, and consequent effects, of $PGF_{2\alpha}$. It is possible that administration of $PGF_{2\alpha}$ *in vivo* may have direct effects upon the corpus luteum via receptor-mediated actions that are potentiated by other indirect $PGF_{2\alpha}$ effects. $PGF_{2\alpha}$ has known vasoconstrictive properties, and injected $PGF_{2\alpha}$ has been shown to reduce blood flow to the corpus luteum [29], which may result in a localized metabolic acidosis in which the acidic milieu could facilitate increased binding of $PGF_{2\alpha}$ to luteal receptors. This hypothesis can be tested in experiments in which

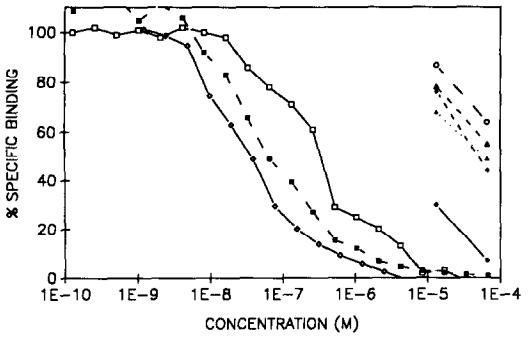

Fig. 6. Displacement curves of $PGF_{2\alpha}$ versus miscellaneous modifications (high affinity potencies relative to $PGF_{2\alpha}$ in brackets; see Materials and Methods for details). Key: (◇) $PGF_{2\alpha}$ [100%]; (■) $PGF_{3\alpha}$ [25.2 ± 3.6%]; (□) $PGF_{1\alpha}$ [8.4 ± 3.3%]; (●) 13,14-dihydro-15-keto-PGD_2 [< 0.1%]; (◆) 13,14-dihydro-15-keto-PGE_2 [< 0.1%]; (▲) 6-keto-PGE_1 [< 0.1%]; (△) 15-keto-PGE_2 [< 0.1%]; and (○) 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$ [< 0.1%].

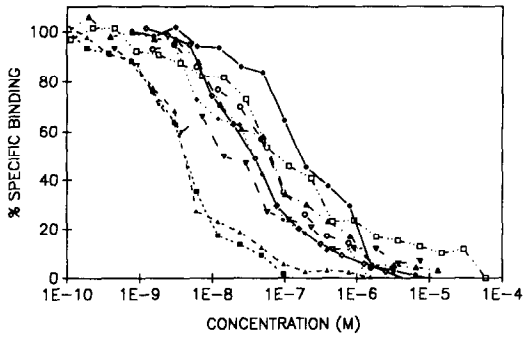

Fig. 7. Displacement curves of $PGF_{2\alpha}$ versus aryl analogs (high affinity potencies relative to $PGF_{2\alpha}$ in brackets; see Materials and Methods for details). Key: (▲) 17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ [756 ± 460%]; (■) 16-phenoxy-17,18,19,20-tetranor-$PGF_{2\alpha}$ [440 ± 222%]; (△) (15*S*)-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ methyl ester [93.2 ± 24.3%]; (○) (15*R*)-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ [61.5 ± 15.6%]; (●) 17-phenyl-PGE_2 [43.3 ± 7.3%]; (▼) $PGF_{2\alpha}$ [100%]; (▽) 15-cyclohexyl-16,17,18,19,20-pentanor-$PGF_{2\alpha}$ [45.9 ± 12.5%]; (□) (15*R*)-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ methyl ester [14.0 ± 4.3%]; and (◇) 16-phenyl-17,18,19,20-tetranor-$PGF_{2\alpha}$ [8.7 ± 4.4%]. The natural 15α configuration is 15*S* in phenyl series and 15*R* in phenoxy series.

luteal cells are incubated *in vitro* with $PGF_{2\alpha}$ in the presence of reduced oxygen and pH.

A multitude of modifications of the native $PGF_{2\alpha}$ molecule were possible that did not abolish the capacity of the modified eicosanoid to compete with [^{3}H]$PGF_{2\alpha}$ for luteal binding sites. Several compounds having an aryl group in the lower side-chain were more potent binding competitors than native $PGF_{2\alpha}$. Compounds having other modifications of $PGF_{2\alpha}$ structure, which included other native prostaglandin species and other cyclopentane ring modifications, and altered upper and/or lower side-chains, exhibited decreased capacity to bind to the $PGF_{2\alpha}$ receptor.

The usual 9,11-dihydroxycyclopentane ring of native $PGF_{2\alpha}$ was not inviolate for retention of binding activity. PGE_2 differs from $PGF_{2\alpha}$ by the presence of a 9-keto group, which reduced binding potency to 1.9 ± 0.3%. Further alteration of the prostaglandin E species by saturation of the 5,6-position resulting in PGE_1 further reduced binding potency to 0.5 ± 0.05%. However, in a luteal cell radioreceptorassay at physiological pH, PGE_1 was devoid of potency [22]. PGD_2, which differs from $PGF_{2\alpha}$ by virtue of an 11-keto group, had binding potency relative to $PGF_{2\alpha}$ of 11.6 ± 2.4%. Thus, in our assay, PGD_2 had greater potency than PGE_2, which is at variance with several cross-reactivity studies that were conducted at physiological pH [17]. The PGA or PGB modifications exhibited essentially no binding activity. It was most interesting that binding potency of the analog having the unusual J ring structure (9,10-dehydro,11-keto) approached equipotency with native $PGF_{2\alpha}$. To our knowledge, PGJ_2 has not been evaluated previously for activity in a $PGF_{2\alpha}$ receptor assay. Additionally, the biological significance of PGJ_2 is unknown.

Several modifications on the upper side-chain were evaluated. Saturation of the 5,6-position yielding $PGF_{1\alpha}$ resulted in a compound having binding potency of 8.4 ± 3.3% relative to $PGF_{2\alpha}$. Methyl esterification of the carboxyl terminus on the upper side chain decreased the relative potency of 17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$ by approximately 8-fold, but, importantly, the esterified form still retained potency that was not significantly different from that of $PGF_{2\alpha}$. Esterification of $PGF_{2\alpha}$ was reported in Ref. 22 to result in an analog having 26% binding potency.

The binding potency of 16,16-dimethyl-$PGF_{2\alpha}$ (159.6 ± 63.5%) was not significantly different from that of $PGF_{2\alpha}$. Other modifications in the lower side-chain resulted in reduced potency. The potency of 15-keto-$PGF_{2\alpha}$, 1.4 ± 0.3%, was similar to the 3.5% relative affinity reported in the bovine luteal receptor in one study [22] and lower than the 13.6% reported for the bovine receptor in Ref. 27. Potency of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ in our system was 1.4 ± 0.3%, which was similar to the previously reported 0.6% [27].

Our initial efforts to develop photoaffinity probes took advantage of the biological and binding potencies of analogs of $PGF_{2\alpha}$ having aryl substitutions in the lower side-chain [22, 30, 31]. Consequently, we constructed analogs having azide-substituted phenyl groups in the 17-position and azide-substituted phenoxy groups in the 16-position. Additional hydroxyl substitutions were incorporated to facilitate radioiodination. However, the hydroxy-substituted analogs that we developed had unacceptable binding potencies. For example, the relative binding potency of 17-(2-hydroxy-4-azidophenyl)-18,19,20-trinor $PGF_{2\alpha}$ was approximately 1% of that exhibited by 17-(4-azidophenyl)-$PGF_{2\alpha}$. The displacement curve of 16-(2-hydroxy-4-azidophenoxy)-17,18,19,20-tetranor-$PGF_{2\alpha}$ methyl ester did not appear to be parallel to the curve from $PGF_{2\alpha}$, suggestive of a complex binding phenomenon. The distally placed hydroxyl moiety in 19-hydroxyl $PGF_{2\alpha}$ also exhibited almost no potency, illustrating that a polar hydro-

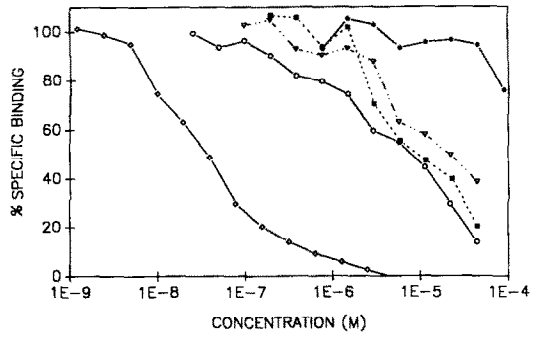

Fig. 8. Displacement curves of $PGF_{2\alpha}$ versus azidophenol analogs (high affinity potencies relative to $PGF_{2\alpha}$ in brackets; see Materials and Methods for details). Key: (◇) $PGF_{2\alpha}$ [100%]; (○) 16-(2-hydroxyl-4-azidophenoxy)-17,18,19,20-tetranor-$PGF_{2\alpha}$ methyl ester [0.2 ± 0.004%]; (■) 17-(2-hydroxyl - 4 - azidophenyl) - 18,19,20 - trinor - $PGF_{2\alpha}$ [0.1 ± 0.01%]; (▽) (15*S*)-16-(2-hydroxyl-4-azidophenoxy)-17,18,19,20-tetranor-$PGF_{2\alpha}$ methyl ester [0.4 ± 0.6%]; and (●) 16-(3-hydroxyl-5-azidophenoxy)-17,18,19,20-tetranor-$PGF_{2\alpha}$ [< 0.1%]. All compounds had natural 15α configuration (15*S* in phenyl series; 15*R* in phenoxy series) except as noted.

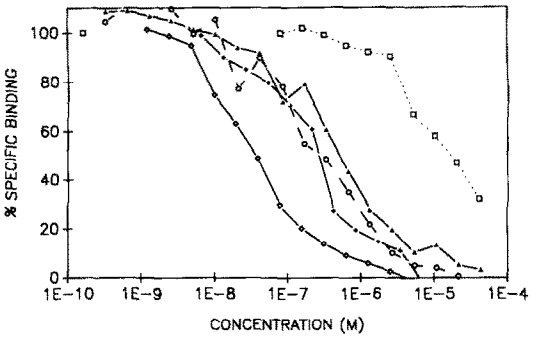

Fig. 9. Displacement curves of $PGF_{2\alpha}$ versus azidophenyl analogs (high affinity potencies relative to $PGF_{2\alpha}$ in brackets; see Materials and Methods for details). Key: (◇) $PGF_{2\alpha}$ [100%]; (◆) (15*S*)-17-(4-azidophenyl)-18,19,20-trinor-$PGF_{2\alpha}$ [10.0 ± 3.4%]; (○) (15*S*)-17-(3-iodo-4-azidophenyl-18,19,20-trinor-$PGF_{2\alpha}$ [4.5 ± 0.54%]; (▲) (5*E*, 15*S*)- and (5*Z*,15*S*)-17-(3-iodo-4-azidophenyl)-18,19,20-trinor-$PGF_{2\alpha}$ [3.4 ± 0.43%]; and (□) 5(6)-iodo-17-(4-azidophenyl)-18,19,20-trinor-$PGF_{2\alpha}$ as a mixture of 5*E* and 5*Z* isomers [0.2 ± 0.03%].

philic hydroxyl group on the lower side-chain of $PGF_{2\alpha}$ analogs had a catastrophic effect on receptor binding potency.

The biological potencies of 17-(4-azidophenyl)-$PGF_{2\alpha}$ or 17-(3-iodo-4-azidophenyl)-$PGF_{2\alpha}$ have not been investigated. However, these compounds retain promising binding affinities, and efforts are currently underway to synthesize [^{125}I]-17-(3-iodo-4-azidophenyl)-$PGF_{2\alpha}$, which may prove to be useful as a $PGF_{2\alpha}$-receptor photoaffinity probe.

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REFERENCES

1. Horton EW and Poyser NL, Uterine luteolytic hormone: A physiological role for prostaglandin $F_{2\alpha}$. *Physiol Rev* **56**: 595–651, 1976.
2. Diekman MA, O'Callaghan P, Nett TM and Niswender GD, Effect of prostaglandin $F_{2\alpha}$ on the number of LH receptors in ovine corpora lutea. *Biol Reprod* **19**: 1010–1013, 1978.
3. McClellan MC, Abel JH Jr and Niswender GD, Function of lysosomes during luteal regression in normally cycling and $PGF_{2\alpha}$-treated ewes. *Biol Reprod* **16**: 499–512, 1977.
4. Pharriss BB, Wyngarden LJ and Gutknecht GD, Biological interaction between prostaglandins and luteotropins in the rat. In: *Gonadotropins* (Ed. Rosemberg E), pp. 121–129. Lost Altos, CA, 1968.
5. Marsh J, The effect of prostaglandins on the adenyl cyclase of the bovine corpus luteum. *Ann NY Acad Sci* **180**: 416–425, 1971.
6. Weston PG and Hixon JE, Effects of *in vivo* prostaglandin $F_{2\alpha}$ administration on *in vitro* progesterone synthesis by bovine corpora lutea. *Biol Reprod* **22**: 259–268, 1980.
7. Fitz TA, Mock EJ, Mayan MH and Niswender GD, Interactions of prostaglandins with subpopulations of ovine luteal cells. II. Inhibitory effects of $PGF_{2\alpha}$ and protection by PGE_2. *Prostaglandins* **28**: 127–138, 1984.
8. Wilks JW, Forbes KK and Norland JF, Prostaglandins and *in vitro* ovarian progestin biosynthesis. *Prostaglandins* **3**: 427–437, 1973.
9. Behrman HR, Luborsky-Moore JL, Pang CY, Wright K and Dorflinger LJ, Mechanisms of $PGF_{2\alpha}$ action in functional luteolysis. In: *Advances in Experimental Medicine and Biology* (Eds. Channing CP, Marsh J and Sadler WA), Vol. 112, pp. 557–571. Plenum Press, New York, 1979.
10. Thomas JP, Dorflinger LJ and Behrman HR, Mechanism of the rapid antigonadotropic action of prostaglandins in cultured luteal cells. *Proc Natl Acad Sci USA* **75**: 1334–1338, 1978.
11. Riley JCM and Carlson JC, Calcium-regulated plasma membrane rigidification during corpus luteum regression in the rat. *Biol Reprod* **32**: 77–82, 1985.
12. Raymond V, Leung PCK and Labrie F, Stimulation by $PGF_{2\alpha}$ of phosphatidic acid-phosphatidylinositol turnover in rat luteal cells. *Biochem Biophys Res Commun* **116**: 39–46, 1983.
13. Leung PC, Minegishi T, Ma F, Zhou F and Ho-Yuen B, Induction of polyphosphoinositide breakdown in rat corpus luteum by prostaglandin $F_{2\alpha}$. *Endocrinology* **119**: 12–18, 1986.
14. Davis JS, Weakland LL, Weiland DA, Farese RV and West LA, Prostaglandin $F_{2\alpha}$ stimulates phosphatidylinositol 4,5-bisphosphate hydrolysis and mobilizes intracellular Ca^{2+} in bovine luteal cells. *Proc Natl Acad Sci USA* **84**: 3728–3732, 1987.
15. Powell WS, Hammarstrom S and Samuelsson B, Prostaglandin $F_{2\alpha}$ receptor in ovine corpora lutea. *Eur J Biochem* **41**: 103–107, 1974.
16. Bayley H, *Photogenerated Reagents in Biochemistry and Molecular Biology*. Elsevier, New York, 1983.
17. Robertson RP, Characterization and regulation of prostaglandin and leukotriene receptors: An overview. *Prostaglandins* **31**: 395–411, 1986.
18. Kawada K, Dolence EK, Morita H, Kometani T, Watt DS, Balapure A, Fitz TA, Orlicky DJ and Gerschenson

LE, Prostaglandin photoaffinity probes: Synthesis and biological activity of azide-substituted 16-phenoxy- and 17-phenyl-$PGF_{2\alpha}$ prostaglandin. *J Med Chem* **32**: 256–264, 1989.
19. Fitz TA, Mayan MH, Sawyer HR and Niswender GD, Characterization of two steroidogenic cell types in the ovine corpus luteum. *Biol Reprod* **27**: 703–711, 1982.
20. Patterson MK Jr, Measurement of growth and viability of cells in culture. In: *Methods in Enzymology* (Eds. Jakoby WB and Pastan IH), Vol. 58, pp. 141–152. Academic Press, New York, 1979.
21. Munson PJ and Rodbard D, LIGAND: A versatile computerized approach for characterization of ligand-binding systems. *Anal Biochem* **107**: 220–239, 1980.
22. Kimball FA, Lauderdale JW, Nelson NA and Jackson RW, Comparison of luteolytic effectiveness of several prostaglandin analogs in heifers and relative binding affinity for bovine luteal prostaglandin binding sites. *Prostaglandins* **12**: 985–995, 1976.
23. Andersen NH, Imamoto S, Subramanian N, Picker DH, Ladner DW, De B, Tynan SS, Eggerman TL, Harker LA, Robertson RP, Oien HG and Rao CV, Molecular basis for prostaglandin potency. III. Tests of the significance of the "hairpin conformation" in biorecognition phenomena. *Prostaglandins* **22**: 841–856, 1981.
24. Powell WS, Hammarstrom S and Samuelsson B, Occurrence and properties of a prostaglandin $F_{2\alpha}$ receptor in bovine corpora lutea. *Eur J Biochem* **56**: 73–77, 1975.
25. Rotondi A and Knazek RA, A rapid method for the measurement of prostaglandin receptors. *Prostaglandins Leukotrienes Med* **9**: 45–53, 1982.
26. Oien HG, Mandel LR, Humes JL, Taub D, Hoffsommer RD and Kuehl FA Jr, Structural requirements for the binding of prostaglandins. *Prostaglandins* **9**: 985–995, 1975.
27. Mattioli M, Galeati G and Seren E, Characteristics of receptors for prostaglandin $F_{2\alpha}$ in bovine and equine corpora lutea. *Prostaglandins Leukotrienes Med* **11**: 259–268, 1983.
28. Rao CV and Mitra S, Subcellular distribution of prostaglandin and gonadotropin receptors in bovine corpora lutea. *Biochem Biophys Res Commun* **76**: 636–643, 1977.
29. Niswender GD, Reimers TJ, Diekman MA and Nett TM, Blood flow: A mediator of ovarian function. *Biol Reprod* **14**: 64–81, 1976.
30. Powell WS, Hammarstrom S, Samuelsson B, Miller WL, Sun FF, Fried J, Lin CH and Jarabak J, Interactions between prostaglandin analogues and a receptor in bovine corpora lutea. Correlation of dissociation constants with luteolytic potencies in hamsters. *Eur J Biochem* **59**: 271–276, 1975.
31. Binder D, Bowler J, Brown ED, Crossley NS, Hutton J, Senior M, Slater L, Wilkinson P and Wright NCA, 16-Aryloxyprostaglandins. A new class of potent luteolytic agent. *Prostaglandins* **6**: 87–90, 1974.