COMPARISON OF LUTEOLYTIC EFFECTIVENESS OF SEVERAL PROSTAGLANDIN ANALOGS IN HEIFERS AND RELATIVE BINDING AFFINITY FOR BOVINE LUTEAL PROSTAGLANDIN BINDING SITES

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ABSTRACT

The relative binding affinities for both the prostaglandin (PG)E₁ and PGF₂α specific bovine luteal binding sites were determined for five PGE and fourteen PGF derivatives and analogs. Relative binding affinity was determined in vitro using membranes prepared from bovine corpora luteal (CL) obtained from the slaughterhouse. The parent structure of the analog was a dominant feature in determining the affinity for the respective PG binding site. Luteolysis was determined in cattle following intramuscular injection of various doses of prostaglandin once between days 6 and 14 after estrus and measuring CL regression by o- varian palpation per rectum, interval between injection and return to estrus and duration of the subsequent estrous cycle. A dose which was luteolytic was established for each of eight PGF-type compounds, and a dose which was not luteolytic was also established. There appeared to be limited association between the relative affinity for the PGF₂α specific site in vitro and the estimated luteolytic dose range of these PGF analogs when tested in cattle. Differences in in vivo luteolytic potency for the compounds tested could not be explained by differences in binding affinity. Differences in metabolism and absorption may also be important in the determination of in vivo potency.

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Exogenous prostaglandin (PG)F₂α induced luteal regression in cattle when administered after day 5 of the estrous cycle (see review, 1). Luteal function was reduced as determined by decreased corpus luteum size and decreased serum progesterone and return to estrus in about three days. Regression of the bovine corpus luteum during the normal estrous cycle is mediated locally by a uterine luteolytic mechanism (2,3). The possibility that PGF₂α is a physiological uterine luteolytic agent has been studied extensively in sheep (see review, 4). Specific prostaglandin E₁ and F₂α binding sites have been demonstrated in ovine (5) and bovine (6-11) corpora lutea (Cl). The specificity of the binding interaction and the apparent dissociation constants suggest that physiological levels of prostaglandins could be bound to luteal binding sites.

We have described specific PGE₁ and PGF₂α binding sites in bovine corpora lutea obtained from slaughter house animals and have compared this binding with the luteolytic effects of these prostaglandins in vivo (11). In the present investigation, the in vitro relative binding affinity has been described for a number of prostaglandin analogs for either the PGE₁ or PGF₂α specific binding site. Prostaglandin F₂α, seven PGF₂α analogs, esters and ethers, PGF₁α and one PGF₁α analog, and PGE₁, tested for in vitro binding affinity were tested also for luteolytic effectiveness in cattle during the estrous cycle. The estimated luteolytic dose range of these compounds in vivo was compared to the respective relative binding affinity in vitro.

MATERIAL AND METHODS

In vitro relative binding affinity:

Bovine luteal particulate fractions were prepared as previously described (11). Briefly, frozen corpora lutea obtained from both pregnant and non-pregnant cattle at a local slaughter house were homogenized in S-P-I buffer (0.25M sucrose-0.02M phosphate buffer, pH 7.4, plus 10 μg/ml indomethacin). The high speed (100,000 g x 1 hr) pellet was prepared, resuspended in S-P-I and stored frozen at -80°C until use. Relative binding affinities were determined by incubating aliquots (100 μl) of particulate fraction added to 0.2 ml S-P-I buffer containing ³H-PGE₁ (1.9 ng/ml, 89.5 Ci/mM) or ³H-PGF₂α (1.0 ng/ml, 178 Ci/mM) with or without added unlabelled test compounds. The various prostaglandins were tested at 7 levels at a concentration range of 1.0-1000 ng/ml. Compounds were prepared and diluted with absolute ethanol and stored at -20°C. The alcoholic addition comprised 1% of the final sample volume and similar amounts of absolute ethanol were added to the control (no unlabelled compound added) tubes. All test compounds were assayed in triplicate and compared to the effect of unlabelled PGE₁ or PGF₂α in the same assay. Incubations were routinely 4 hr at 22°C. Removal of the unbound PG was accomplished using 1.0 ml 2.5% charcoal in 0.1% gelatin-0.02M phosphate buffer, pH 7.4 (11). Assay blanks (100 μl 0.5% gelatin-phosphate buffer) and totals (1.0 ml buffer instead of charcoal) were incubated in each assay. Siliconized glassware was used.
throughout. The results were analyzed by parallel line assay. The mean assay blank value ($n=6$) was subtracted from individual experimental values and the mean $^3$H-PG bound of each sample ($n=3$) was graphed versus the log-dose of the unlabelled compound. Only that portion of the curve showing significant parallelism with the control (PGE$_1$ or PGF$_{2\alpha}$) was considered in the analysis; a minimum of three dose levels was used in all cases. Single determinations of the affinities of two prostaglandins relative to the reference standard must differ by three-fold for them to be declared statistically different.

In vivo luteolytic measurements:

Luteolytic potency of various prostaglandins was tested in beef and dairy cattle. Luteolytic effectiveness was measured initially with two doses, one dose anticipated to be effective, and one dose anticipated to be non-effective. The doses were 60 mg and 15 mg for PGF$_{1\alpha}$ and PGF$_{2\alpha}$, methyl ester but were 25 mg and 5 mg for all other prostaglandins evaluated (Table 1). If the 5 mg dose was luteolytic, lower doses were tested, with the exception of PGF$_{2\alpha}$, methyl ester (Table 1). Three to 13 animals were assigned randomly to each prostaglandin-dose groups. Animals were injected once intramuscularly (IM) between days 6 and 14 of the estrous cycle (estrus = day 0). Animals were observed for estrous behavior both morning and evening. Ovaries were palpated per rectum on the day of injection to ascertain presence of a CL, on day 3, 4 or 5 after injection to determine either development of a new CL or regression of the CL that failed to respond to the prostaglandin. A dose of a given prostaglandin was declared effective only if greater than 80 percent of treated animals had their CL regressed by days 2 to 5 after injection, were detected in estrus during days 2 and 5 after injection, and had a normal estrous cycle subsequent to estrus detected following treatment with prostaglandin.

Prostaglandins administered to cattle were dissolved in 95% ethanol (maximum concentration of 6% to 40% in the final solution), diluted with sterile distilled water, and pH adjusted to neutrality with sodium bicarbonate where necessary.

Synthesis of 16,16-dimethyl-17-phenyl-18,19,20-trinor-PGF$_{2\alpha}$ (VIII):

Treatment of 2,2-dimethyl-3-phenylpropionic acid (12) with thionyl chloride afforded the corresponding acid chloride which was allowed to react with the lithio derivative of dimethyl methylphosphate (13) to give dimethyl 3,3-dimethyl-2-oxo-4-phenylbutylphosphonate (I), mp 49-51°. The remaining steps of the synthesis followed published procedures (14-16) with some modifications and included: (a) condensation of the potassium enolate of I with 3α-benzoyloxy-2β-formyl-5α-hydroxy-1α-cyclopentancarboxylic acid, γ-lactone (III) (17) to give IV (m.p. 103-104°); b) reduction of IV with Zn(BH$_4$)$_2$ and separation of the desired (R)-alcohol (less polar) from the (S)-alcohol by chromatography of the mixture on E. Merck silica gel with 50-75% ethyl acetate in Skellysolve B; c) formation of the diol V (K$_2$CO$_3$ and methanol, then HCl); (d) formation of the corresponding bis (tetrahydropyranyl ether); followed by (e) standard elaboration of the carboxy side chain (14). The product VIII was homogeneous by tlc and exhibits key nmr and mass spectral peaks.
Table I
Comparison of Relative Binding Affinities of Prostaglandins for Bovine Luteal Particulate Fractions and Effective Luteolytic Dose Range in Heifers During the Estrous Cycle

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Affinity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Effective Dose Range (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>^H-PGE&lt;sub&gt;1&lt;/sub&gt;</td>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>1.4 (1.7-1.1)</td>
<td>100</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt;, methyl ester</td>
<td>0.2 (0.3-0.2)</td>
<td>26.3 (34.3-20.2)</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;1α&lt;/sub&gt;</td>
<td>0.1 (0.1-0.1)</td>
<td>4.3 (5.2-3.7)</td>
</tr>
<tr>
<td>cis-Δ&lt;sup&gt;4&lt;/sup&gt;-PGF&lt;sub&gt;1α&lt;/sub&gt;</td>
<td>0.3 (0.4-0.3)</td>
<td>11.0 (13.0-9.3)</td>
</tr>
<tr>
<td>11-deoxy-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>0.8 (0.9-0.6)</td>
<td>23.7 (29.9-18.7)</td>
</tr>
<tr>
<td>13,14-dihydro-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>0.1 (0.1-0.1)</td>
<td>3.5 (4.8-2.4)</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt;, 15-methyl ether (15S)-15-methyl-PGF&lt;sub&gt;2α&lt;/sub&gt;-THAM</td>
<td>1.6 (1.8-1.4)</td>
<td>49.8 (58.5-42.3)</td>
</tr>
<tr>
<td>16,16-dimethyl-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>5.1 (5.5-4.8)</td>
<td>78.0 (96.7-63.6)</td>
</tr>
<tr>
<td>16,16-dimethyl-17 phenyl-18,19,20-trinor-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>0.3 (0.4-0.3)</td>
<td>40.6 (49.5-33.6)</td>
</tr>
<tr>
<td>16-methyl-16-phenoxy-18,19,20-trinor-PGF&lt;sub&gt;2α&lt;/sub&gt;, methyl ester</td>
<td>2.4 (2.6-2.2)</td>
<td>7.0 (8.3-5.9)</td>
</tr>
<tr>
<td>16-phenoxy-17,18,19,20-tetranor-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>6.8 (8.4-5.3)</td>
<td>121.9 (163.3-91.3)</td>
</tr>
<tr>
<td>16-(m-trifluoromethylphenoxy)-17,18,19,20-tetranor-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>0</td>
<td>69.3 (92.5-51.6)</td>
</tr>
<tr>
<td>17-phenyl-18,19,20-trinor-PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>0.1 (0.1-0.1)</td>
<td>86.8 (100.5-74.6)</td>
</tr>
<tr>
<td>17-phenyl-18,19,20-trinor-PGF&lt;sub&gt;1α&lt;/sub&gt;</td>
<td>0</td>
<td>23.0 (28.7-18.4)</td>
</tr>
<tr>
<td>PGE&lt;sub&gt;1&lt;/sub&gt;</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>cis-Δ&lt;sup&gt;4&lt;/sup&gt;-PGE&lt;sub&gt;1&lt;/sub&gt;, methyl ester</td>
<td>6.5 (7.6-5.4)</td>
<td>0.1</td>
</tr>
<tr>
<td>11-deoxy-PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>73.4 (81.9-65.9)</td>
<td>0</td>
</tr>
<tr>
<td>(15S)-15-methyl-PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>77.4 (89.1-67.0)</td>
<td>1.5 (2.5-0.9)</td>
</tr>
<tr>
<td>16,16-dimethyl-PGE&lt;sub&gt;1&lt;/sub&gt;</td>
<td>149.2 (166.2-133.9)</td>
<td>0</td>
</tr>
<tr>
<td>17-phenyl-18,19,20-trinor-PGE&lt;sub&gt;1&lt;/sub&gt;</td>
<td>51.4 (56.9-46.3)</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>95% confidence interval in parentheses  
<sup>b</sup>NT = not tested
in agreement with its structure (see Figure 1).

**Synthesis of 16-methyl-16-phenoxy-18,19,20-trinor-PGF\textsubscript{2\alpha}, methyl ester (IX)**

Dimethyl 3-methyl-1-oxo-3-phenoxybutylphosphonate (II) was prepared from 2-methyl-2-phenoxypropionic acid in a manner analogous to that described above for the preparation of I. Condensation of the sodium enolate of II with III afforded VI (m.p. 145-147°). Reduction of VI with \( \text{Zn(BH}_4\text{)}_2 \), separation of isomers, and removal of the benzoate group from the less-polar \( \text{R} \) isomer afforded VII. The remaining steps in the synthesis were carried out as described above (see 18 also) for the preparation of VIII and the product was isolated as the methyl ester IX. The homogeneity of IX was established by tlc and it exhibits the expected nmr and mass spectral peaks for the assigned structure.

Syntheses of other prostaglandin analogs studied have been described (15,16,19-23).

**RESULTS**

The relative affinities are reported for several prostaglandin derivatives and analogs for the \( ^3\text{H}-\text{PGE}_1 \) and \( ^3\text{H}-\text{PGF}_2\alpha \) luteal binding sites (Table 1). In general, \( \text{PGE}_1 \) and \( \text{PGF}_2\alpha \) do not displace each other effectively, nor do their respective analogs displace each other effectively. In \( \text{PGE}_1 \), methylation at carbon 16 (16,16-dimethyl-\( \text{PGE}_1 \)) caused enhanced binding affinity compared to \( \text{PGE}_1 \). Modification at carbon 15 and 17, by substitution of a methyl group for the hydrogen at carbon 15 in \( \text{PGE}_1 \) or the 17-phenyl-18,19,20-trinor modification in \( \text{PGE}_1 \) resulted in moderately decreased relative binding affinity. Similarly, removal of the \( \text{11α-hydroxyl} \) (11-deoxy-\( \text{PGF}_2\alpha \)) had little effect on binding affinity. The introduction of a cis-4,5-double bond (cis-\( \Delta^4 \)-\( \text{PGF}_{1\alpha} \), methyl ester) significantly decreased the affinity for the \( ^3\text{H}-\text{PGE}_1 \) binding site. The binding of \( ^3\text{H}-\text{PGE}_1 \) could not be inhibited significantly by \( \text{PGF}_2\alpha \) analogs tested (see Table 1).

The amount of \( ^3\text{H}-\text{PGF}_2\alpha \) binding depended on changes in the \( \text{PGF}_2\alpha \) parent structure. Modification at carbon 16 caused either enhanced (16-phenoxy-17,18,19,20-tetranor-\( \text{PGF}_2\alpha \)) or similar (16,16-dimethyl-\( \text{PGF}_2\alpha \)) binding affinity compared to \( \text{PGF}_2\alpha \). Similarly, the 17-phenyl-18,19,20-trinor modification did not significantly change the binding affinity. Modifications of \( \text{PGF}_2\alpha \) at carbon 15, by substitution of a methyl ether group for the 15-hydroxyl or a methyl group for the hydrogen at carbon 15 (15\text{-methyl-}\( \text{PGF}_2\alpha \)-THAM) resulted in binding affinities approximately 25-50% of \( \text{PGF}_2\alpha \). This was similar to the effect of removal of the carbon 11 functional group (11-deoxy-\( \text{PGF}_2\alpha \)). Combination of 16,16-dimethyl substitution with either the 17-phenyl-18,19,20-trinor or the 16-phenoxy-17,18,19,20-tetranor (16-methyl-16-phenoxy-18,19,20-trinor-\( \text{PGF}_2\alpha \), methyl ester) modifications resulted in decreased binding affinity compared to any of these changes alone. The relative affinity of 16-phenoxy-17,18,19,20-tetranor-\( \text{PGF}_2\alpha \) appeared to be attenuated by the addition of the \( \text{m-trifluoromethyl} \) group to the phenyl ring (Table 1). The cis-4,5-double bond (cis-\( \Delta^4 \)-\( \text{PGF}_{1\alpha} \)) or the saturated 5,6-double bond (\( \text{PGF}_{1\alpha} \)) resulted in significantly lower bind-
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Figure 1

Intermediates in the Synthesis of
16,16-Dimethyl-17-phenyl-18,19,20-trinor-PGF$_{2\alpha}$ (VIII) and
16-Methyl-16-phenoxy-18,19,20-trinor-PGF$_{2\alpha}$, Methyl Ester (IX)

I

$\text{CH}_2\cdot\text{C(CH}_3)_2\cdot\text{CO}\cdot\text{CH}_2\cdot\text{PO(OCH}_3)_2$

II

$\text{O}\cdot\text{C(CH}_3)_2\cdot\text{CO}\cdot\text{CH}_2\cdot\text{PO(OCH}_3)_2$

III, $R_1=\text{C}_6\text{H}_5\cdot\text{CO}$, $R_2=\text{CHO}$

IV, $R_1=\text{C}_6\text{H}_5\cdot\text{CO}$, $R_2=\text{CH}$

V, $R_1=\text{H}$, $R_2=\text{CH}$

VI, $R_1=\text{C}_6\text{H}_5\cdot\text{CO}$, $R_2=\text{CH}$

VII, $R_1=\text{H}$, $R_2=\text{CH}$

VIII

IX

990

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ing affinities. The 13,14-dihydro metabolites of PGF$_{2\alpha}$ also had minimal affinity for the $^3$H-PGF$_{2\alpha}$ binding site. Incubation of PGF$_{2\alpha}$, methyl ester with non-specific hog liver esterase prior to the binding reaction increased the binding affinity 4-fold, suggesting that hydrolysis of the methyl ester improves competition for the binding site in vitro (data not shown).

The test system employed to determine the luteolytic potency of prostaglandin analogs estimated the upper and lower limits for the minimal effective dose. Based on this test system, one analog, 17-phenyl-18,19,20-trinor-PGF$_{2\alpha}$, was more potent than PGF$_{2\alpha}$ in causing luteolysis in cattle. PGE$_1$ was less potent than PGF$_{2\alpha}$ and two compounds, PGF$_{2\alpha}$, methyl ester and PGF$_{1\alpha}$, were probably less potent than PGF$_{2\alpha}$. The five other analogs tested were effective in the same dose range as that used for PGF$_{2\alpha}$ and are judged equipotent based on these tests.

DISCUSSION

Comparison of effectiveness of certain prostaglandins as luteolytic agents in cattle and their respective relative binding affinity for the PGF$_{2\alpha}$ specific binding site suggests there is limited association between these two factors. It is clear that binding affinity alone cannot account for the luteolytic effectiveness of these prostaglandins when compared to PGF$_{2\alpha}$. Several factors influence the in vivo potency of a compound, such as its half-life both systematically in the circulation and locally in the tissue, differences in cell permeability rates, as well as its specificity (relative affinity) at the target organ. The 17-phenyl-18,19,20 trinor modification of PGF$_{2\alpha}$ or PGE$_1$ reduced by 50% the PG-15-hydroxy dehydrogenase activity as compared to PGF$_{2\alpha}$ (24). The 16,16-dimethyl substituted PG analogs were not substrates for this dehydrogenase (15). Similar decreases in the rate of 15-dehydrogenation occur with PGF$_{2\alpha}$, 15-methyl ether and 13,14-dihydro-PGF$_{2\alpha}$. The difference in potency between the PGF$_{2\alpha}$, methyl ester and PGF$_{2\alpha}$ (free acid) suggests that these compounds are metabolized differently. The increased relative binding affinity of the free acid compared to the methyl ester suggests that the ester linkage is cleaved prior to binding in vivo. The results of the present study indicate that the dose required to produce luteolysis in cattle varies for certain prostaglandin analogs. The effectiveness of the respective prostaglandin appears to be modified by the relative ease of metabolism of the analog as well as influenced by the relative binding affinity.

A previous report compared the dissociation constants for some prostaglandin analogs and the PGF$_{2\alpha}$ specific binding site in bovine corpora lutea and the antifertility potencies of these same compounds tested in pregnant hamsters (25). These values appeared positively associated if the rates of metabolism by prostaglandin 15-hydroxy dehydrogenase were taken into account. The most active analogs with regard to both affinity for the binding site and hamster luteolytic potency were 17-phenyl-18,19,20-trinor-PGF$_{2\alpha}$ and (15S)-15-methyl-PGF$_{2\alpha}$. The high luteolytic potency of the 17-phenyl analog in pregnant hamsters is similar to
that seen in cattle in vivo. However, the relative binding affinity of the analog in vitro is probably not different from that of PGF$_{2\alpha}$, suggesting that decreased metabolism is responsible for the increased potency.

PGF$_{2\alpha}$ has been shown to be luteolytic in cattle when administered any day after the fifth day following estrus (see review, 1,3). The range of doses of PGF$_{2\alpha}$ required to cause luteolysis depends on the method of administration and ranges from 5 mg intrauterine (26) to 25 mg intramuscular (27). No reports are available which report precisely the minimum effective intramuscular dose for cattle. There have been few comparisons of the luteolytic effects of PGF$_{2\alpha}$ with other prostaglandins in cattle. A twenty-five milligram intramuscular injection of PGE$_1$ did not affect estrous cycle length or decrease plasma progesterone when administered to heifers on day 11-13 of the cycle (11). A one milligram intramuscular injection into cattle of the analog dl-16-(p-fluorophenoxy)-17,18,19,20-tetranor-PGF$_{2\alpha}$ (I.C.I. 79,939) resulted in luteolysis followed by fertile estrus (28). Plasma progesterone concentrations decreased by 6 hrs after treatment and basal concentrations were reached by 24 hrs post-treatment (29). The changes in plasma concentrations of progesterone following treatment with either PGF$_{2\alpha}$ or the PGF$_{2\alpha}$ analog were similar, and also similar to that occurring prior to natural estrus (29). These results suggest that PGF$_{2\alpha}$ and PGF$_{2\alpha}$ analogs may act by the same mechanism as luteolytic agents in cattle. The results of the present study indicate that certain structural modifications of the PGF$_{2\alpha}$ parent alter the effective dosage and retain the desired activity.
REFERENCES


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