Prostaglandin moieties that determine receptor binding specificity in the bovine corpus luteum

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This study provided a pharmacological evaluation of prostaglandin binding to bovine luteal plasma membrane. It was found that [3H]PGF2α, [3H]PGE2, [3H]PGE, and [3H]PGD2, all bound with high affinity to luteal plasma membrane but had different specificities. Binding of [3H]PGF2α and [3H]PGD2 was inhibited by non-radioactive PGF2α (IC50 values of 21 and 9 nmol l−1, respectively), PGD2 (35 and 21 nmol l−1), and PGE2 (223 and 81 nmol l−1), but not by PGE1 (> 10000 and 5616 nmol l−1). In contrast, [3H]PGE was inhibited by non-radioactive PGE2 (14 nmol l−1) and PGE1 (7 nmol l−1), but minimally by PGD2 (2316 nmol l−1) and PGF2α (595 nmol l−1). Binding of [3H]PGE was inhibited by all four prostaglandins, but slopes of the dissociation curves indicated two binding sites. Binding of [3H]PGE1 was inhibited, resulting in low IC50 values, by pharmacological agonists that are specific for EP3 receptor and possibly EP2 receptor. High affinity binding of [3H]PGF2α required a C15 hydroxyl group and a C1 carboxylic acid that are present on all physiological prostaglandins. Specificity of binding for the FP receptor depended on the C9 hydroxyl group and the C5/C6 double bond. Alteration of the C11 position had little effect on affinity for the FP receptor. In conclusion, there is a luteal EP receptor with high affinity for PGE2, PGE1, agonists of EP1 receptors, and some agonists of EP2 receptors. The luteal FP receptor binds PGF2α, PGD2 (high affinity), and PGE2 (moderate affinity) but not PGE1 due to affinity determination by the C9 and C5/C6 moieties, but not the C11 moiety.

Introduction

Prostaglandins have a variety of regulatory functions in the corpus luteum. It is well established that PGF2α is the primary initiator of luteolysis in many species, including all species of ruminant investigated (reviewed by Knickerbocker et al., 1988). The physiological role of type E prostaglandins is not clearly defined, but PGE, or PGE1, have been implicated in regulation of the duration of the oestrous cycle, luteal progesterone production, luteolysis, and maternal recognition of pregnancy (Kimball and Lauderdale, 1975; Gimenez and Henricks, 1983).

Cloning studies show that prostaglandin receptors are members of the seven transmembrane domain receptor superfamily (reviewed by Negishi et al., 1995). Although prostaglandin receptors are similar in amino acid sequence and structure (Negishi et al., 1995) and prostaglandins are similar in chemical structure, it has been reported that prostaglandins only bind and activate specific receptors (Coleman et al., 1994). The current nomenclature for prostaglandin receptors corresponds to the naturally occurring prostanoid that binds to that receptor with the highest affinity (for example PGD2 binds to the DP receptor, PGF2α binds to the FP receptor; Coleman et al., 1995). There are multiple receptor subtypes that bind PGE2 (EP1, EP2, EP3, reviewed by Ushikubi et al., 1995) and specific pharmacological agonists have been used to differentiate EP receptor subtypes. Although a number of studies have evaluated prostaglandin binding in the corpus luteum of various species, no study has used specific pharmacological agonists to investigate the PGE receptor subtype in the corpus luteum. Accordingly, the first two experiments in this study were designed to characterize the specificity of prostaglandin binding and to identify the EP receptor subtypes in the bovine corpus luteum. Experiment 3 was designed to provide novel information on the precise moieties on the PGF2α molecule that determine specificity of binding to the FP receptor.

Materials and Methods

Reagents

Misoprostol and SC-19220 were gifts from G. D. Searle (Skokie, IL). Sulprostone and M&B 28,767 were donated by Schering (Berlin) and Rhône-Poulenc Rorer Ltd (Dagenham).
respectively. All other non-radioactive prostaglandins and
prostaglandin derivatives were obtained from Cayman
Chemical (Ann Arbor, MI). [H]PGD2, [H]PGE2, [H]PGE1,
and [H]PGF2a were purchased from New England
Nuclear/Dupont (Boston, MA). Gelatin was purchased
from Difco Laboratories (Detroit, MI). All other chemicals
were obtained from Sigma Chemical Company (St Louis, MO).

Tissue homogenization

Mid-cycle corpora lutea were selected from bovine ovaries
obtained from an abattoir. Minced corpora lutea were
suspending in tissue homogenization buffer (10 mmol trizma
hydrochloride 1 M, 20 mmol sucrose 1 M, 1 mmol CaCl2
1 M, 1 mmol MgCl2 1 M, 0.02% (w/v) Na2S2O3 pH 7.0). Tissue was
homogenized on ice using an ultraturrax homogenizer
(Tekmar Company, Cincinnati, OH) and then with a
Wheaton glass tissue grinder (Fisher Scientific, Pittsburgh,
PA). The plasma membrane fraction was obtained by
centrifuging the homogenized tissues three times at 3000 g
for 5 min at 4°C to remove tissue debris. The supernatant
was then centrifuged at 30000 g for 30 min at 4°C. The plasma
membrane pellet was resuspended in tissue homogenization buffer. Protein content was measured using
the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules,
CA). Aliquots were stored at -70°C. Immediately before
receptor binding studies, the tissue was thawed on ice and
then centrifuged for 30 min at 30000 g. The pellet was
resuspended in assay buffer (20 mmol 2-[N-morpholino]-ethanesulfonic acid (MES) 1 M, 10 mmol CaCl2 1 M, 10 mmol
MgCl2 1 M, 0.02% (w/v) Na2S2O3 0.1% (w/v) gelatin, pH 6.0). A single luteal plasma membrane preparation was used for
Expts 1 and 2 and a different preparation was used for Expt 3.

Radioreceptor assays

A time and temperature course of the binding assay
described below was run initially to determine a single
time point and temperature at which [H]PGD2, [H]PGE2,
[H]PGE1, and [H]PGF2a showed stable binding. Three
incubation temperatures (4°C, 20°C, and 37°C) and seven time
points (0.5, 1, 2, 4, 8, 12 and 24 h) were examined. Binding was
stable for all four [H]prostaglandins at 4°C between 12 and 24
h. All other radioreceptor assays were run at 4°C for 20 h.

Assays were conducted in low-binding 96-well microtitre
plates (Fisher Scientific, Pittsburgh, PA) in 100 μl volume using
200 μg plasma membrane protein. Tritated prostaglandins
(3 nmol 1 M) were inhibited by various concentrations of
non-radioactive prostaglandins or prostaglandin agonists
(1-10000 nmol 1 M) diluted in assay buffer. Samples were
incubated on a microtitre plate shaker (Fisher Scientific,
Pittsburgh, PA) for 20 h at 4°C. Free prostaglandins were
separated from bound prostaglandins by rapid filtration
with a cell harvester. Plasma membrane protein (containing
bound prostaglandin) was collected on glass fibre filter
paper (Schleicher & Schuell Inc., Keene, NH) and washed
thoroughly with wash buffer (20 mmol MES 1 M, 1 mmol
CaCl2 1 M, 1 mmol MgCl2 1 M, 0.02% (w/v) Na2S2O3 pH 6.0) at 4°C.

Samples were placed in BioSafe II scintillation fluid
(Beckman Co., Fullerton, CA), vortexed for 1 min, and stored
in a dark area for at least 12 h. Samples were placed in a
scintillation counter for 3 min to determine the amount of
radioactivity. Inhibition curves were constructed for each
radioactive and each non-radioactive prostaglandin.

Scatchard analysis was used to determine the number and
affinity of FP and EP receptors by displacement of
[H]PGE2, and [H]PGF2a by non-radioactive PGE and PGF2a,
respectively (Scatchard, 1949). The procedures were similar
to those for the inhibition curve assays. The assays were
conducted in 96-well microtitre plates in 100 μl using 106.5
and 213.0 μg plasma membrane protein (15 and 30 mg initial
weight of corpora lutea, respectively). [H]PGE2, and
[H]PGF2a (3 nmol 1 M) binding was inhibited by increasing
amounts (0.06-6 nmol 1 M) of cold PGE1 and PGF2a,
respectively.

Non-radioactive PGD2, PGE2, PGE1 or PGF2a (10 μmol 1 M
approximately equivalent to 3.5 μg prostaglandin ml-1)
was incubated for 20 h (4°C) with bovine luteal plasma membrane in
conditions similar to those for the radioreceptor assay to
test for degradation of PGF2a or conversion of prostaglandins
to PGF2a. Plasma membrane was removed by centrifugation
at 30000 g for 30 min at 4°C, and the assay buffer from each
sample was analysed for PGF2a by radioimmunoassay
(Townson and Pate, 1994).

Statistical analysis

The data from the inhibition curves in Expt 1 were applied
to a logistic model to estimate the concentration at which
50% binding occurred and the slope of the curve at that
point. The non-linear regression routine in S-plus software
(Mathsoft Inc., Seattle, WA) was used. The model assumes
that the response will start at 100% for low concentrations
of non-radioactive prostaglandins and decrease toward 0% as
the concentration of non-radioactive prostaglandin
increases. The following form of the model was used.

\[ p = 1 - \left\{ 1 / \left[ 1 + \exp \left( (x - \theta_1) / \theta_2 \right) \right] \right\} \]

where \( x = \log_{10} (\text{concentration of non-radioactive prostaglandin}) \)
and \( p = \text{percentage } [H] \text{prostaglandin bound/100.} \) With
this parameterization, \( \theta_1 \) is the x value on the log scale where
the curve reaches half its height (\( p = 0.5 \) or the IC50 value), and \( \theta_2 \)
is a scale parameter, measured in the units of the x-axis, and
is related to the steepness of the curve. The derivative of
the curve (slope) at 50% is \( 1/4(x \times \theta_2) \). The absolute value of \( \theta_2 \)
is the distance on the x-axis over which the response decreases
from 73.1 to 50%, and from 50 to 26.9%, due to the symmetry
of the curve. This parameter is inversely related to the slope
of the curve; a large \( \theta_2 \) reflects a relatively flat curve, whereas
a small \( \theta_2 \) corresponds to a steep rapidly descending curve.
Within each curve, the variability appeared homogeneous.
Among the curves there were differences in variability as
reflected in the widths of the confidence intervals for
the parameter estimates. Nominal 95% confidence intervals for
\( \theta_1 \) and \( \theta_2 \) were computed using each parameter estimate and
its standard error (Tables 1 and 2) to facilitate comparisons
across multiple curves.
Table 1. Inhibition (50%) of radioactive prostaglandins by non-radioactive prostaglandins bound to bovine luteal plasma membrane

<table>
<thead>
<tr>
<th>Radioactive prostaglandin</th>
<th>PGD&lt;sub&gt;2&lt;/sub&gt;</th>
<th>PGE&lt;sub&gt;1&lt;/sub&gt;</th>
<th>PGE&lt;sub&gt;2&lt;/sub&gt;</th>
<th>PGF&lt;sub&gt;2α&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>[³H]PGD&lt;sub&gt;2&lt;/sub&gt;</td>
<td>21</td>
<td>5616</td>
<td>81</td>
<td>9</td>
</tr>
<tr>
<td>(18,24)</td>
<td>(4799,6572)</td>
<td>(68,97)</td>
<td>(8,11)</td>
<td></td>
</tr>
<tr>
<td>[³H]PGE&lt;sub&gt;1&lt;/sub&gt;</td>
<td>2316</td>
<td>14</td>
<td>7</td>
<td>595</td>
</tr>
<tr>
<td>(1703,3148)</td>
<td>(11,17)</td>
<td>(6,9)</td>
<td>(453,782)</td>
<td></td>
</tr>
<tr>
<td>[³H]PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>242</td>
<td>81</td>
<td>9</td>
<td>66</td>
</tr>
<tr>
<td>(172,339)</td>
<td>(58,112)</td>
<td>(7,12)</td>
<td>(49,88)</td>
<td></td>
</tr>
<tr>
<td>[³H]PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>35</td>
<td>&gt;10000</td>
<td>223</td>
<td>21</td>
</tr>
<tr>
<td>(27,45)</td>
<td>(176,282)</td>
<td>(17,26)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = 3 experiments performed in triplicate. IC<sub>50</sub> (nmol l<sup>-1</sup>) = mean. Confidence intervals (95%) are shown in parentheses (lower limit, upper limit). For calculation of IC<sub>50</sub> and standard errors the data were in log units. Values were transformed to the inverse log of IC<sub>50</sub> to provide IC<sub>50</sub> values, thus the confidence intervals appear asymmetrical.

Table 2. Scale parameter (θ, = distance on x-axis from 73.1 to 50% bound) for displacement by non-radioactive prostaglandins of radioactive prostaglandins bound to bovine luteal plasma membrane

<table>
<thead>
<tr>
<th>Radioactive prostaglandin</th>
<th>PGD&lt;sub&gt;2&lt;/sub&gt;</th>
<th>PGE&lt;sub&gt;1&lt;/sub&gt;</th>
<th>PGE&lt;sub&gt;2&lt;/sub&gt;</th>
<th>PGF&lt;sub&gt;2α&lt;/sub&gt;</th>
<th>Row average</th>
</tr>
</thead>
<tbody>
<tr>
<td>[³H]PGD&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.48</td>
<td>0.46</td>
<td>0.60</td>
<td>0.50</td>
<td>0.51</td>
</tr>
<tr>
<td>(0.42,0.54)</td>
<td>(0.39,0.53)</td>
<td>(0.53,0.66)</td>
<td>(0.44,0.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[³H]PGE&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.74</td>
<td>0.43</td>
<td>0.49</td>
<td>0.65</td>
<td>0.57</td>
</tr>
<tr>
<td>(0.60,0.88)</td>
<td>(0.33,0.52)</td>
<td>(0.39,0.58)</td>
<td>(0.54,0.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[³H]PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.99</td>
<td>0.92</td>
<td>0.60</td>
<td>0.76</td>
<td>0.82</td>
</tr>
<tr>
<td>(0.85,1.13)</td>
<td>(0.79,1.06)</td>
<td>(0.50,0.70)</td>
<td>(0.65,0.88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[³H]PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td>0.53</td>
<td>0.36</td>
<td>0.46</td>
<td>0.37</td>
<td>0.43</td>
</tr>
<tr>
<td>(0.44,0.62)</td>
<td>(0.19,0.53)</td>
<td>(0.38,0.55)</td>
<td>(0.30,0.45)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The curve is symmetrical so the scale parameter describes the slope between 26.9 and 73.1%. The estimated slope of the curve at 50% is 1/(4 x θ). 

n = 3 experiments performed in triplicate. Values are predicted scale parameters from logistic model fit. Confidence intervals (95%) are shown in parentheses (lower limit, upper limit).

Results

Time and temperature effects on binding

All four tritiated prostaglandins demonstrated stable binding at 4°C between 12 and 24 h. This was not necessarily the highest point of binding for each individual prostaglandin. However, under these conditions, all [³H]prostaglandins bound with high affinity to bovine luteal plasma membrane.

Experiment 1: prostaglandin receptors in the corpus luteum

The number of [³H]PGE<sub>2α</sub> molecules specifically bound to 200 µg plasma membrane protein was 6.7 ± 0.5 x 10<sup>10</sup> (mean ± SEM). Binding of [³H]PGF<sub>2α</sub> (Fig. 1a, Table 1) was inhibited by non-radioactive PGE<sub>2α</sub> and PGD<sub>2</sub> with similar affinity (IC<sub>50</sub> = 21 and 35 nmol l<sup>-1</sup>, respectively). Cold PGE<sub>2</sub> displaced [³H]PGF<sub>2α</sub> binding with lower affinity (IC<sub>50</sub> = 223 nmol l<sup>-1</sup>), whereas PGE<sub>2</sub> did not inhibit the binding of [³H]PGF<sub>2α</sub> to luteal plasma membrane (IC<sub>50</sub> > 10 µmol l<sup>-1</sup>).

The number of [³H]PGD<sub>2</sub> molecules specifically bound to 200 µg plasma membrane protein was 4.6 ± 0.6 x 10<sup>10</sup> (mean ± SEM). Similar to [³H]PGF<sub>2α</sub> binding, [³H]PGD<sub>2</sub> binding (Fig. 1b, Table 1) was inhibited by PGD<sub>2</sub> (IC<sub>50</sub> = 21 nmol l<sup>-1</sup>), PGE<sub>2α</sub> (IC<sub>50</sub> = 9 nmol l<sup>-1</sup>) and PGE<sub>2</sub> (IC<sub>50</sub> = 81 nmol l<sup>-1</sup>). Non-radioactive PGE<sub>2</sub> only displaced [³H]PGD<sub>2</sub> at a high concentration (IC<sub>50</sub> = 5616 nmol l<sup>-1</sup>). The number of [³H]PGE<sub>2</sub> molecules specifically bound to 200 µg plasma membrane protein was 1.6 ± 0.1 x 10<sup>10</sup> (mean ± SEM). Both PGE<sub>2</sub> and PGE<sub>2α</sub> displaced the binding of [³H]PGE<sub>2</sub> to luteal plasma membrane with similar affinity (IC<sub>50</sub> = 14 and 7 nmol l<sup>-1</sup>, respectively). At much higher concentrations, PGF<sub>2α</sub> (IC<sub>50</sub> = 595 nmol l<sup>-1</sup>) and PGD<sub>2</sub> (IC<sub>50</sub> = 2316 nmol l<sup>-1</sup>) inhibited [³H]PGE<sub>2</sub> binding (Table 1, Fig. 1c).

The number of [³H]PGE<sub>2</sub> molecules specifically bound to 200 µg plasma membrane protein was 2.9 ± 0.2 x 10<sup>10</sup> (mean ± SEM). Binding of [³H]PGE<sub>2</sub> was inhibited with a low concentration of PGE<sub>2</sub> (IC<sub>50</sub> = 9 nmol l<sup>-1</sup>). Higher concentrations of PGF<sub>2α</sub> (IC<sub>50</sub> = 66 nmol l<sup>-1</sup>), PGE<sub>2</sub> (IC<sub>50</sub> = 81 nmol l<sup>-1</sup>) and PGD<sub>2</sub> (IC<sub>50</sub> = 242 nmol l<sup>-1</sup>) were required to displace [³H]PGE<sub>2</sub> (Fig. 1d, Table 1).

The scale parameters relate to the slope between 27% and 73% (Table 2) and were greatest for displacement of [³H]PGE<sub>2</sub>. This is consistent with the relatively flat displacement lines for [³H]PGE<sub>2</sub> (Fig. 1d). Two other scale parameters that were relatively large were for the displacement of [³H]PGE<sub>2</sub> by PGD<sub>2</sub> and PGF<sub>2α</sub>. The remaining scale parameters were between 0.36 and 0.60. From these results, the decision was
made to use [3H]PGE1 to characterize further the EP receptors, since the [3H]PGE2 binding results were compromised by the apparent binding of PGE1 to both EP and FP receptors.

The radioimmunoassay did not detect an increase in the concentration of PGF2α in the assay buffer after 20 h incubation of high concentrations of non-radioactive PGD2, PGE1, PGE2, or PGF2α with bovine luteal plasma membrane, indicating that PGD2, PGE, and PGE2 were not being converted to PGF2α (data not shown). There appeared to be some crossreactivity of PGD2 and PGE2 with the PGF2α antibody, since a value of 0.1–1.0 ng PGF2α ml⁻¹ was detected for PGD2 and PGE2 before incubation with plasma membrane. The PGF2α concentration detected in the assay buffer before and after 20 h incubation of PGD2 or PGE2 with bovine luteal plasma membrane decreased by 60% and 97%, respectively, indicating possible prostaglandin degradation.

Scatchard analysis showed a single high affinity binding site for [3H]PGE1 and [3H]PGF2α (Fig. 2). The dissociation constants (Kd) for the EP receptor were 7.6 and 9.2 nmol l⁻¹ for 106.7 and 213.0 μg luteal plasma membrane protein, respectively (equivalent to 15 and 30 mg of initial corpus luteum). The concentration of EP receptors (Bmax) was 437 and 507 amol μg⁻¹ protein for 213.0 and 106.5 μg plasma membrane protein, respectively (Fig. 2). The dissociation constant for the FP receptor was 11.4 nmol l⁻¹ for 216 μg plasma membrane protein and 11.1 nmol l⁻¹ for 106.7 μg plasma membrane protein. The concentration of FP receptors in the plasma membrane was 2915 and 2986 amol μg⁻¹ protein for 213.0 and 106.5 μg plasma membrane protein, respectively (Fig. 2).

**Experiment 2: EP receptor subtype determination**

A variety of relatively specific agonists was used to inhibit [3H]PGE1 binding to determine the EP receptor subtype in the bovine corpus luteum. M&B 28,767 (EP receptor agonist), 11-deoxy PGE1 (EP2 > EP1), 16,16-dimethyl PGE2, (EP3 > EP1 > EP2), 17-phenyl trinor PGE2 (EP2 > EP3), and sulprostone (EP3 > EP2) displaced [3H]PGE1 binding with relatively high affinity (IC50 = 3, 8, 9, 17 and 23 nmol l⁻¹, respectively) (Table 3). Misoprostol (EP2 > EP3) inhibited [3H]PGE1 binding at a higher concentration (IC50 = 82 nmol l⁻¹), whereas SC-19220 (EP1) did not displace [3H]PGE1 binding (IC50 > 10000 nmol l⁻¹) (Table 3). Similar to PGF2α, fluprostenol (FP receptor agonist) was not able to inhibit [3H]PGE1 binding effectively (IC50 = 6167 nmol l⁻¹).

**Experiment 3: determination of essential sites for PGF2α binding to FP receptor**

Alterations to C1 of PGF2α markedly decreased the ability of prostaglandin derivatives to inhibit [3H]PGF2α binding. The IC50 value for PGF2α dimethyl amide was > 10 μmol l⁻¹ and for PGF2α isopropyl ester was 383 ± 27 nmol l⁻¹ (Table 4).
Similarly, oxidation at C15 (15-keto PGF₂α) substantially altered binding of [³H]PGF₂α (IC₅₀ = 667 ± 145 nmol l⁻¹) (Table 4).

Reduction at C9 (PGE₂) had a moderate effect on [³H]PGF₂α binding (IC₅₀ = 157 ± 27 nmol l⁻¹). Similarly, changes at the double bond between C5 and C6 decreased inhibition of [³H]PGF₂α binding (5-trans PGF₂α, PGF₁α; IC₅₀ = 61 ± 5, 153 ± 22, respectively; Table 4).

Changes at other sites in PGF₂α had a minimal effect on inhibition of [³H]PGF₂α binding. PGD₂ and 11-deoxy PGF₂α (changes at C11) had IC₅₀ values of 27 ± 3 and 37 ± 4 nmol l⁻¹, respectively. Hydrogenation C13 and C14 produced a slight change in [³H]PGF₂α inhibition (13,14-dihydro PGF₂α; IC₅₀ = 21 ± 5 nmol l⁻¹). Dehydration at C17 and C18 (PGF₃α) resulted in an IC₅₀ value of 39 ± 5 nmol l⁻¹. Changes beyond C16 had little effect on binding (PGF₃α, fluprostrenol, cloprostenol; IC₅₀ = 39 ± 5, 34 ± 6 and 5 ± 2 nmol l⁻¹, respectively; Table 4).

**Discussion**

This study provides a pharmacological analysis of prostaglandin binding in the bovine corpus luteum. The systematic approach used in these experiments provided insight into the nature of prostaglandin binding in the corpus luteum.

It is apparent that PGE₂ binds to both FP and EP receptors. This is indicated by the inhibition of [³H]PGF₂α binding by PGE₂ and, conversely, PGF₂α inhibition of [³H]PGE₂ binding. In addition, the flattened slopes for inhibition of [³H]PGE₂ by PGD₂, PGF₂α, and PGE₁ indicated that there are two different binding sites with different affinities for [³H]PGE₂. This is also supported by the observation that the number of [³H]PGE₂ molecules (2.9 × 10¹⁰) bound to the bovine luteal plasma membrane was higher than the number of [³H]PGE₂ (1.6 × 10¹⁰) molecules bound. Therefore, studies in which PGE₂ is used for either binding or physiological evaluations must be considered at least partially compromised because of this crossreactivity. For example, treatment of ovine large luteal cells with PGE₂ caused an increase in free intracellular calcium (Wiepz et al., 1993) with an ED₅₀ value (168 nmol l⁻¹) that corresponds closely to the affinity of the FP receptor for PGE₂ observed in this study and in other investigations (Wiepz et al., 1992).

In contrast, PGE₁ appeared to interact with only the EP receptor and not the FP receptor (this study; Rao, 1974; Kimball and Lauderdale, 1975; Wiepz et al., 1992). This was demonstrated by the large IC₅₀ values for inhibition of [³H]PGD₂ or [³H]PGF₂α by PGE₁. Similarly, very high concentrations of PGD₂ and PGF₂α were required to inhibit binding of [³H]PGE₂. Several studies have demonstrated that PGE₂ and PGE₃ can have very different physiological effects on the corpus luteum. For example, in ewes in which the ovarian pedicle was treated with PGF₂α there was a decrease in luteal weight and serum progesterone concentration that could be restored to values observed in vehicle-treated ewes by simultaneous administration of PGE₁ but not PGE₂ (Reynolds et al., 1981). In addition, the luteal phase of ewes was extended by administration of PGE₁ into the uterine horn ipsilateral but not contralateral to the corpus luteum (Huie et al., 1981). Likewise, administration of high doses of PGE₁ in pseudopregnant rats delayed luteolysis, whereas administration of high doses of PGE₂ accelerated the onset of luteolysis (Weems et al., 1979). Therefore, researchers interested in studying actions mediated through EP receptors in the corpus luteum should consider using PGE₂, or other specific EP receptor agonists, rather than PGE₁, because of the interaction of PGE₂ with FP as well as EP receptors.
### Table 3. Prostaglandin E₁ agonists, structure, inhibition constants and specificity

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Chemical structure</th>
<th>IC₅₀ (nmol l⁻¹)</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₁</td>
<td><img src="image1" alt="Chemical structure" /></td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>M&amp;B 28,767</td>
<td><img src="image2" alt="Chemical structure" /></td>
<td>3</td>
<td>EP₃</td>
</tr>
<tr>
<td>PGE₂</td>
<td><img src="image3" alt="Chemical structure" /></td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>11-deoxy PGE₁</td>
<td><img src="image4" alt="Chemical structure" /></td>
<td>8</td>
<td>EP₂ &gt; EP₃</td>
</tr>
<tr>
<td>16,16-dimethyl PGE₂</td>
<td><img src="image5" alt="Chemical structure" /></td>
<td>9</td>
<td>EP₂ &gt; EP₁ &gt; EP₃</td>
</tr>
<tr>
<td>17-phenyl trinor PGE₂</td>
<td><img src="image6" alt="Chemical structure" /></td>
<td>17</td>
<td>EP₁ &gt; EP₃</td>
</tr>
<tr>
<td>Sulprostone</td>
<td><img src="image7" alt="Chemical structure" /></td>
<td>23</td>
<td>EP₃ &gt; EP₁</td>
</tr>
<tr>
<td>Misoprostol</td>
<td><img src="image8" alt="Chemical structure" /></td>
<td>82</td>
<td>EP₂ &gt; EP₃</td>
</tr>
<tr>
<td>SC-19220</td>
<td><img src="image9" alt="Chemical structure" /></td>
<td>&gt; 10000</td>
<td>EP₁</td>
</tr>
</tbody>
</table>

Arrows indicate the sites at which the analogues differ from PGE₁.
Superscripts indicate references that identify EP receptor specificity: ¹Negishi et al., 1993; ²Regan et al., 1994; ³Li et al., 1993; ⁴Jumblatt and Paterson, 1991; ⁵Coleman et al., 1994; ⁶Watabe et al., 1993; ⁷Yang et al., 1994; ⁸Honda et al., 1993; ⁹Coleman et al., 1985.
Table 4. Prostaglandin F<sub>2α</sub> analogues, structure and inhibition constants

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Chemical structure</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (nmol l&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Differs from PGF&lt;sub&gt;2α&lt;/sub&gt; at</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td><img src="Image1" alt="Chemical structure of PGF&lt;sub&gt;2α&lt;/sub&gt;" /></td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt; dimethyl amide</td>
<td><img src="Image2" alt="Chemical structure of PGF&lt;sub&gt;2α&lt;/sub&gt; dimethyl amide" /></td>
<td>&gt; 10000</td>
<td>C1</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;2α&lt;/sub&gt; isopropyl ester</td>
<td><img src="Image3" alt="Chemical structure of PGF&lt;sub&gt;2α&lt;/sub&gt; isopropyl ester" /></td>
<td>383</td>
<td>C1</td>
</tr>
<tr>
<td>5-trans PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td><img src="Image4" alt="Chemical structure of 5-trans PGF&lt;sub&gt;2α&lt;/sub&gt;" /></td>
<td>61</td>
<td>C5/C6 bond</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;10&lt;/sub&gt;</td>
<td><img src="Image5" alt="Chemical structure of PGF&lt;sub&gt;10&lt;/sub&gt;" /></td>
<td>153</td>
<td>C5/C6 bond</td>
</tr>
<tr>
<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td><img src="Image6" alt="Chemical structure of PGE&lt;sub&gt;2&lt;/sub&gt;" /></td>
<td>157</td>
<td>C9</td>
</tr>
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<td>PGD&lt;sub&gt;2&lt;/sub&gt;</td>
<td><img src="Image7" alt="Chemical structure of PGD&lt;sub&gt;2&lt;/sub&gt;" /></td>
<td>27</td>
<td>C11</td>
</tr>
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<td>11-deoxy PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td><img src="Image8" alt="Chemical structure of 11-deoxy PGF&lt;sub&gt;2α&lt;/sub&gt;" /></td>
<td>37</td>
<td>C11</td>
</tr>
<tr>
<td>13,14-dihydro PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td><img src="Image9" alt="Chemical structure of 13,14-dihydro PGF&lt;sub&gt;2α&lt;/sub&gt;" /></td>
<td>21</td>
<td>C13/C14 bond</td>
</tr>
<tr>
<td>15-keto PGF&lt;sub&gt;2α&lt;/sub&gt;</td>
<td><img src="Image10" alt="Chemical structure of 15-keto PGF&lt;sub&gt;2α&lt;/sub&gt;" /></td>
<td>667</td>
<td>C15</td>
</tr>
<tr>
<td>PGF&lt;sub&gt;3α&lt;/sub&gt;</td>
<td><img src="Image11" alt="Chemical structure of PGF&lt;sub&gt;3α&lt;/sub&gt;" /></td>
<td>39</td>
<td>C17/C18 bond</td>
</tr>
<tr>
<td>Fluprostenol</td>
<td><img src="Image12" alt="Chemical structure of Fluprostenol" /></td>
<td>34</td>
<td>C17 tail</td>
</tr>
<tr>
<td>Cloprostenol</td>
<td><img src="Image13" alt="Chemical structure of Cloprostenol" /></td>
<td>5</td>
<td>C17 tail</td>
</tr>
</tbody>
</table>

Arrows indicate the sites at which the analogues differ from PGF<sub>2α</sub>. 

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receptors. This may be less of a problem in tissues that contain small numbers of FP receptors (Tsai et al., 1998). The obvious problem with using PGE is that PGE appears to be the physiological ligand for EP receptors in most cases. For example, the uterine concentrations of PGE are approximately tenfold greater than those of PGE, (Wilson et al., 1972). Thus, it is unclear whether crossreactivity of PGE, with the FP receptor is involved in normal physiological events or is a pharmacological phenomenon.

Although PGD, binds with high affinity to luteal plasma membrane, it is likely that PGD, is binding to the FP receptor rather than a specific DP receptor. In preliminary experiments, a specific agonist for the DP receptor (BW-245C) was unable to displace either [H]PGD or [H]PGF binding in bovine luteal plasma membrane (IC > 10 μmol l⁻¹; L. E. Anderson and M. C. Wiltbank, unpublished). Wipf et al. (1992) reported that PGD, inhibits [H]PGF₆-binding in the ovine corpus luteum. Binding studies in mammalian COS cells transfected with human FP receptor also demonstrated that PGD, inhibits binding of [H]PGF₆ (Abramovitz et al., 1994). Cloning and expression of the bovine FP receptor also showed crossreactivity with PGD, (Sakamoto et al., 1994). Higher concentrations of PGD, were required to displace [H]PGF₆ binding to mouse and rat FP receptors (Lake et al., 1994; Sugimoto et al., 1994), indicating a species difference in receptor crossreactivity. Interestingly, the DP receptor does not appear to bind either PGE, or PGF, in mice or humans (Hirata et al., 1994; Boie et al., 1995).

Experiment 2 was designed to determine the subtype of luteal EP receptor using pharmacological agonists. The finding that M&B 28,767 displaced [H]PGE, with high affinity indicates that an EP₁ type receptor is present (Negishi et al., 1993). In addition, 16,16-dimethyl PGE, preferentially binds EP₁ receptors, although this compound may have non-selective binding to all EP receptors in some species (Jumblatt and Paterson, 1991; Li et al., 1993). Furthermore, high affinity binding of sulprostone indicates that most luteal prostaglandin E binding is due to EP₁ receptors (Watabe et al., 1993; Yang et al., 1994). The EP₁ receptor agonists available crossreact with the EP₁ receptor, thus the presence of an EP₁ receptor could not be confirmed. The high affinity binding of 11-deoxy PGE₁ (Regan et al., 1994) indicates that an EP₂ receptor may be present. However, misoprostol bound with lower affinity, as may be expected for an EP₂ receptor. Although several EP receptor agonists bind to the EP₁ receptor, most of these also bind to EP₃ receptors. The lack of binding of the EP₁ selective agonist SC-19220 (Coleman et al., 1985) indicates that EP₁ receptors may not be present in the bovine corpus luteum, although low solubility of this compound may cause aberrant results. The slope of the inhibition curves indicated that the affinity of only one receptor was being inhibited by each of the agonists. Thus, these results are consistent with the luteal prostaglandin E receptor being of the EP₁ receptor subtype. An EP₂ receptor may also be present, but could not be demonstrated directly. Further studies with EP receptor-specific antibodies would provide more direct evidence for these conclusions.

Experiment 3 provided insight into the moieties involved in PGF₂ₐₐ binding. The C9 position was the only moiety that altered PGF₂ₐₐ binding, and was unique to the PGF₂ₐₐ molecule compared with the other common prostaglandins. Changing the C9 from hydroxyl to ketone (PGF₂₉ to PGE₂) increased the IC₅₀ value by more than tenfold. In contrast, alteration of the other unique site for PGF₂₉, C11, had a minimal effect on PGF₂₉ binding. This could be demonstrated by changing the C11 site to a ketone (PGD₁) or by complete removal of the hydroxyl group (11-deoxy PGF₂₉). Interestingly, removal of the C11 hydroxyl from the PGE₂ molecule also had little effect on PGE₂ binding. Thus, it appears that C9 offers the primary site for luteal prostaglandin receptor specificity, whereas C11 moieties appear to have minimal effect on bovine luteal EP or FP receptor binding.

The three groups that were important for PGF₂₉ binding but were not specific to the PGF₂₉ molecule were C₁, C₅/C₆, and C₅. Replacement of the C₁ carboxylic acid with large groups (dimethyl amide or isopropyl ester) almost eliminated binding to the receptor. Unfortunately, there were no compounds available that altered only the nature of the carboxylic acid without the addition of large residues. It has been proposed that the C₁ carboxylic acid forms a Schiff base with conserved arginine in the seventh transmembrane domain (residue 291 of the bovine FP receptor; Sakamoto et al., 1994) and stabilizes binding of all prostaglandins to their receptors.

The C₅/C₆ double bond is important for PGF₂₉ binding, as indicated by the finding that removal of this double bond (PGF₁₉) increased the IC₅₀ value by more than tenfold, and isomerization of the bond from cis to trans conformation (5-trans PGF₂₉) significantly increased the IC₅₀ value. Although this bond is not unique to the PGF₂₉ molecule, it appears to have a major impact on receptor specificity. This is demonstrated by the fact that PGE₂ (C₅/C₆ double bond intact) has a moderate IC₅₀ value (157 nmol l⁻¹), whereas PGE₂ (C₅/C₆ double bond removed) caused virtually no displacement of [H]PGF₂₉ (> 1000 nmol l⁻¹). The C₅/C₆ double bond did not alter luteal prostaglandin E binding, as shown by high affinity binding of PGE or PGE₂ as well as a number of different agonists that contained (sulprostone, 16,16-dimethyl PGE₂, 17-phenyl trino PGF₂) or did not contain (M&B 28,767, 11-deoxy PGE₂) the C₅/C₆ double bond.

The hydroxyl in the C₁₅ position appears to be essential for binding because changing this group to a ketone (15-keto PGF₂₉) markedly reduced affinity for the FP receptor. It is possible that the hydroxyl in the C₁₅ position, along with the carboxylic acid at C₁, provide ionic interactions that stabilize PGF₂₉ binding to the FP receptor. There was little effect on PGF₂₉ or prostaglandin E binding or activity when alterations were made in the molecule beyond C₁₇, similar to the findings of other studies (Negishi et al., 1993; Watabe et al., 1993; Yang et al., 1994; Graves et al., 1995). The addition of larger groups, such as phenols, after C₁₇ has been used to increase the half-life for prostaglandins in vivo since these compounds are less susceptible to 15-hydroxy prostaglandin dehydrogenase (Miller et al., 1975; Granström, 1975). Phenyl-substituted PGF₂₉ is at least as potent as PGF₂₉ (Miller et al., 1975; Resul et al., 1993).

In summary, high affinity binding of prostaglandins to
bovine luteal plasma membrane indicated the presence of specific FP and EP receptors. The affinity of different prostaglandins and prostaglandin analogues for these receptors indicated moieties on the prostaglandin molecule that are essential for binding to luteal prostaglandin receptors and that luteal EP receptor is probably of the EP, subtype, although results on the presence of luteal EP receptors are equivocal. The group attached to the C9 position appears to be most critical for determining specificity of luteal FP and EP receptors. A cis double bond at C5/C6 is also critical for luteal FP but not EP receptor binding. Other parts of the prostaglandin molecule, including C11, appear to be minimally important for determination of luteal prostaglandin receptor specificity.

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