Prostaglandin E_2 and $F_{2\alpha}$ Binding Sites in the Bovine Iris Ciliary Body

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Purpose. To determine the regional distribution and selectivity of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) specific binding sites in membrane preparations from bovine iris ciliary body.

Methods. Bovine eyes were obtained fresh from a local abattoir. Whole irides were separated into sphincter muscle, ciliary body, and remaining iris tissue then homogenized in 50 mM tris buffer, pH 7.5, containing cyclooxygenase, protease, and soybean trypsin inhibitors. Membranes were obtained after three-stage high-speed centrifugation then reconstituted in buffer. Aliquot portions were incubated with ^{3}H-PGE_2 or ^{3}H-$PGF_{2\alpha}$ at 37°C for 30 min in a final volume of 500 μl. Competition studies were performed in the presence of up to 1000-fold excess unlabeled ligand. At the end of a 30-min incubation period, free and membrane bound ligand were separated by rapid filtration through a type HA millipore filter preequilibrated with buffer. The radioactivity bound to the membranes retained on the filter was quantitated in a scintillation counter.

Results. The equilibrium dissociation constant and maximum number 3H-PGE_2 saturable binding sites were determined by Scatchard analysis. The data best fit to a single binding site model for all three membrane preparations. The majority of the ^{3}H-PGE_2 specific binding sites were in sphincter muscle (65%), followed by iris (17%), and ciliary body (18%). ^{3}H-$PGF_{2\alpha}$ binding sites were not measurable in either iris or ciliary body and $PGF_{2\alpha}$ was less competitive than PGE_2 in all tissues. The rank order of potency for agonist displacement by both ^{3}H-PGE_2 and ^{3}H-PGF_2 in sphincter muscle membrane was $PGE_2 \rangle PGF_{2\alpha} \rangle PGD_2 \rangle$ Iloprost. It was determined that ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ binding in the bovine sphincter was primarily to EP not FP, DP, or IP prostaglandin receptor types.

Conclusions. The characteristics of PGE_2 and $PGF_{2\alpha}$ specific binding sites in the bovine sphincter correlate with its in vitro contractile response to these prostanoids. The inability of $PGF_{2\alpha}$ to effectively compete with PGE_2 for specific binding sites in the sphincter and lack of high affinity $PGF_{2\alpha}$ specific binding sites in the iris and ciliary body suggests that this prostaglandin may exert its in vivo effects through PGE_2 specific binding sites. Invest Ophthalmol Vis Sci 1993;34:2237–2245.

Prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) have well characterized physiologic and pathophysiologic roles in the eye.[1-5] They elicit receptor-mediated responses including: vasodilation, alteration of intraocular pressure, and the constriction of iris sphincter and ciliary muscles.[5-7]. Recent efforts have been directed at identifying the type and distribution of prostaglandin specific binding sites in ocular tissues.[8-11]. The current nomenclature classifies five types of prostanoid receptors based on their affinity for naturally occurring prostanoids,[12,13] i.e., PGE_2 (EP), $PGF_{2\alpha}$ (FP), PGD_2 (DP), thromboxane (TP), and

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prostacyclin (IP). In addition, the EP receptor has been subclassified into at least three subtypes EP_1, EP_2 and EP_3.[14-16] This nomenclature was developed by testing the functional responses of various tissues to naturally occurring prostanoids and their analogs.[13,15-17] An alternate system of classification was developed based on second messenger generation in response to prostanoid exposure.[18] We have characterized specific prostanoid binding sites in membrane preparations from bovine ocular tissue based on their affinity for selected prostanoid agonists and antagonists.

The characteristics of ocular eicosanoid receptors are only now being examined. PGE_2 receptors in cultured rabbit corneal endothelial cells are reported to be of the EP_2 subtype, linked to activation of adenylate cyclase and important in the maintenance of endothelial morphology.[8] EP_2 receptors apparently mediate breakdown of the rabbit blood–aqueous barrier[9] and activation of EP_3 receptors in the whole iris ciliary body can inhibit adrenergic neurotransmission.[10] $PGF_{2\alpha}$ is believed to contract feline and canine iris sphincter muscle via a distinct FP receptor.[19,20] In the bovine sphincter, PGE_2 is a more potent contractile agent than $PGF_{2\alpha}$[21,22]; however, the receptor responsible for sphincter contraction is unclear. It has been suggested that iris sphincter contractile state is controlled by means of stimulation of a receptor mediated myo-inositol 1,4,5-triphosphate (IP_3) transduction pathway.[23] Stimulation of the FP receptor has been demonstrated to result in IP_3 formation.[16,18,24-26]

We have previously reported the properties of PGE_2-selective binding sites in the membrane preparations of whole bovine iris ciliary body.[11]. In the current study the kinetic characteristics and regional distribution of ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ specific binding sites in membrane preparations of separated bovine sphincter muscle, iris and ciliary body are examined. Our goal is to determine whether both PGE_2 and $PGF_{2\alpha}$ specific binding sites are present, their regional distribution within the iris ciliary body, and, whether our findings can help interpret data from functional studies.

MATERIALS AND METHODS

Membrane Preparation

Fresh bovine eyes were obtained from a local abattoir and transported on ice to the laboratory. Whole iris ciliary body was dissected circumferentially into three component parts: the sphincter muscle, the ciliary body and processes, and the remaining iris tissue. The sphincter was first dissected free then the ciliary body with attached ciliary processes was dissected. The remaining iris consisted of vasculature and dilator muscle with a minor contamination from sphincter muscle and ciliary body fragments. Membranes were prepared from these three isolated tissues. This investigation adhered to the "ARVO Statement for the Use of Animals in Ophthalmic and Vision Research."

The tissues were maintained at 2–4°C and the anatomic identity of separated tissues was verified in histologic sections (not shown). Each tissue was homogenized separately in 50 mM tris buffer, pH 7.5, containing cyclooxygenase (1 μM flurbiprofen), protease (0.43 mM PMSF), and 0.01% soybean trypsin inhibitors. In addition the homogenization buffer also contained 1 mM EGTA. Membranes were obtained after three-stage high-speed centrifugation was done as previously reported.[11] Tissue from 80 eyes yielded 3.1, 4.5, and 9 mg membrane protein[27] from sphincter, iris and ciliary body, respectively.

Radioligand Binding Assay

The homogenization buffer was supplemented with 2.5 mM manganese (Mn^{++}) and 0.2% bovine serum albumin for both competition and saturation studies. Membranes were reconstituted in buffer and aliquot portions containing 50–300 μg of membrane protein were incubated with 8 nM ^{3}H-PGE_2 (200 Ci/mmol) or ^{3}H-$PGF_2\alpha$ (209 Ci/mmol) at 37°C for 30 min in a final volume of 500 μl. Competition studies were performed in the presence of varying concentrations of unlabeled ligand as described previously.[11]. A 1000-fold excess of the unlabeled ligand was employed to define the level of nonspecific binding. Nonspecific binding represented 30–40% of total binding in the sphincter and ciliary body membranes. The iris membranes displayed the highest percentage (70%) of nonspecific binding. At the end of a 30-min incubation period, free and membrane bound ligand were separated by rapid filtration through a type HA millipore filter preequilibrated with buffer. The radioactivity bound to the membranes retained on the filter was quantitated in a scintillation counter. Blank values for radioactivity retained by the filters in the absence of membrane were subtracted from all values.

Scatchard analysis for the determination of maximum number of binding sites (B_{max}) and equilibrium dissociation constant (K_d) was performed in sphincter muscle, iris, and ciliary body preparations using 8 nM ^{3}H-PGE_2 and zero-1000 nM unlabeled ligand.

Data Analysis

Data were analyzed using the analytic portion of the computer software program Sigma Plot Version 4.0 (Jandel Scientific) and EBDA/Ligand (Biosoft). EBDA was used to perform the transformations necessary to analyze the Scatchard data. Sigma Plot was used to fit data by an iterative process to the four-parameter logistic equation. This analysis produced a "best fit" to a

sigmoidal curve. The analysis provided a value for the IC_{50} (concentration of unlabeled ligand that displaced 50% of specifically bound labeled ligand) of the competing ligand and a coefficient of cooperativity describing the relative shape of the sigmoidal function.

Materials

Prostaglandins (Cayman Chemical, Ann Arbor, MI) were made up to a 14.2 mM concentration in 95% ethanol. Working solutions contained less than 0.18% ethanol. BWA868C was donated by Wellcome Research Laboratories, United Kingdom. Iloprost was donated by Schering AG, Germany. Labeled ligands were purchased from New England Nuclear (Boston, MA). All other compounds were obtained from Sigma Chemical Company (St. Louis, MO).

RESULTS

Analysis of PGE_2- and $PGF_{2\alpha}$ Binding Site Data

In a typical experiment, using membrane from separated iris, after subtraction of the filter blanks, we find 5588 DPM (disintegrations per minute) captured on filters from the total binding tubes and 3379 DPM from the nonspecific binding tubes, allowing a maximum of 1708 DPM for specific binding calculations. In sphincter muscle, where the greatest binding occurred, there were 23,122 DPM from the total binding tubes and 6128 from the nonspecific binding tubes leaving 16594 DPM as specific binding. These data are converted to femtomoles (fmol) bound per milligram of membrane protein and analyzed by the Ligand/EBDA binding program for estimation of kinetic parameters. The program allows for the determination of whether the data from one or several saturation studies best fit to a single- or multiple-site model.

^{3}H-PGE_2 saturation data for sphincter, iris, and ciliary body revealed Hill plot values for these tissues of 0.75, 0.81, and 0.88, respectively. The K_d and B_{max} values for sphincter, iris, and ciliary body were: 3.1, 28, and 17.8 nM, and 842, 154, and 82 fmoles bound/mg protein, respectively. The data analysis indicated a best fit to a single-site model for each tissue. $PGF_{2\alpha}$ binding sites in sphincter were not sufficiently saturated by 8nM $PGF_{2\alpha}$ to report meaningful scatchard analysis data. No significant specific $PGF_{2\alpha}$ binding to iris or ciliary body membranes was measured.

These data are summarized in Table 1.

Distribution of PGE_2 Binding Sites

The distribution of PGE_2 specific binding sites within each separated region of the iris-ciliary body was calculated. B_{max} values from Scatchard analysis data were used to determine the percentage of binding sites located within each tissue type. Based on the quantity of membrane protein recovered for each region of the iris ciliary body, the majority of PGE_2 binding sites were determined to be located in the sphincter muscle followed by the ciliary body and the iris (Table 1). Although the sphincter represented 19% of the membrane protein, 65% of the PGE_2 binding sites were located within this tissue. Ciliary body, representing 54% of the recovered membrane protein, contained 18% of the PGE_2 specific binding sites.

Competition Studies

^{3}H-PGE_2 Binding Sites in Sphincter, Iris, and Ciliary Body. The relative selectivity of unlabeled PGE_2 and $PGF_{2\alpha}$ to displace bound 8 nM ^{3}H-PGE_2 is shown in Figure 1. The IC_{50} values for displacement of ^{3}H-PGE_2 specific binding by PGE_2 from membranes were as follows: sphincter, 7.5 nM; iris, 10.5 nM; and ciliary body, 7.9nM. In all three membrane preparations, $PGF_{2\alpha}$ was 30–100-fold less effective than PGE_2 at competing for the ^{3}H-PGE_2 binding site. The IC_{50} values for displacement of ^{3}H-PGE_2 specific binding by $PGF_{2\alpha}$ from membranes were as follows: sphincter, 792 nM; iris, 304 nM; and ciliary body, 608 nM.

^{3}H-$PGF_{2\alpha}$ Binding Sites in Sphincter, Iris, and Ciliary Body. The relative affinities of unlabeled $PGF_{2\alpha}$ or PGE_2 to displace 8 nM ^{3}H-PGE_2 or ^{3}H-$PGF_{2\alpha}$ binding from sites in the sphincter are shown in Figure 2. The IC_{50} values for displacement of ^{3}H-$PGF_{2\alpha}$ were 0.8 nM

TABLE 1. Scatchard Analysis of the Regional Distribution of PGE_2 Binding Sites in Separated Bovine Iris–Ciliary Body Membrane Preparations

		Sphincter	*Iris*	*Ciliary Body*
PGE_2				
nmol/l	K_d	3.1 ± 1.2	28 ± 14	17.8 ± 7.0
fmol bound/mg protein	B_{max}	842 ± 51	154 ± 43	82 ± 14
% of whole ICB membrane protein		19	27	54
fmol bound/tissue (%)		65	17	18

Values are mean ± SEM.

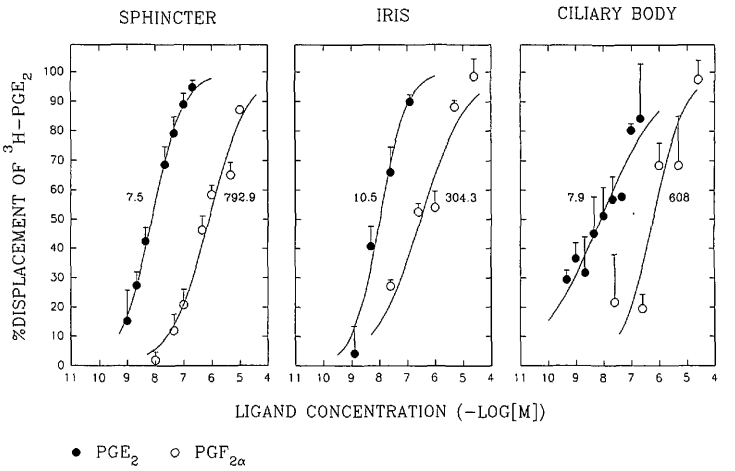

FIGURE 1. Competitive inhibition of 8 nM ^{3}H-PGE$_2$ (200 Ci/mmol) specific binding to bovine sphincter, iris, and ciliary body membranes by unlabeled PGE$_2$ and PGF$_{2\alpha}$. Incubations were performed in a 500 μl final volume, for 30 min at 37.5°C in triplicate, in the presence or absence of various concentrations of unlabeled ligand. 100% specific binding is defined as the amount of bound ^{3}H-PGE$_2$ displaced in the presence 1 μM unlabeled PGE$_2$ (n = 6 for each PGE$_2$ and n = 3 for each PGF$_{2\alpha}$ point represented, values represent the mean ± SEM). Calculated IC$_{50}$ values (nM) are shown next to each curve.

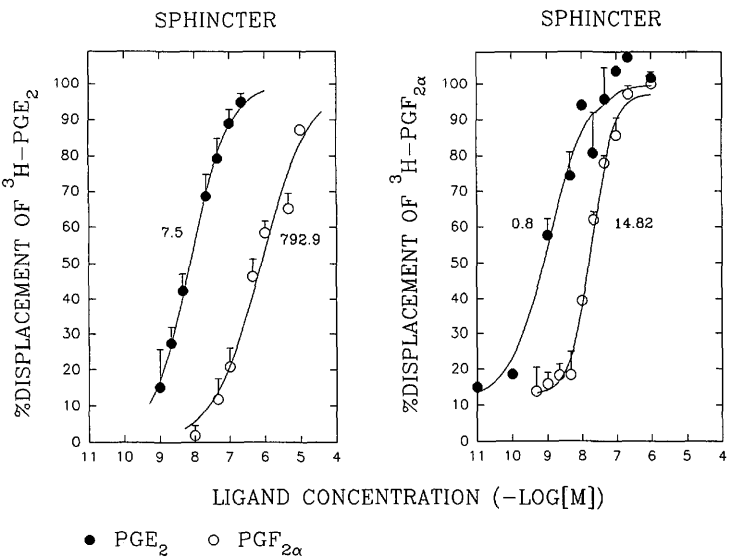

FIGURE 2. Comparison of competitive curves for inhibition of 8 nM ^{3}H-PGE$_{2\alpha}$ (*left panel*) and 8 nM ^{3}H-PGF$_{2\alpha}$ (209 Ci/mmol; *right panel*) specific binding to bovine sphincter muscle membranes by unlabeled PGF$_{2\alpha}$ and PGE$_2$. Incubation conditions were the same as those in Fig. 1 (n = 3 or more for each ligand, values represent the mean ± SEM). Calculated IC$_{50}$ values (nM) are shown next to each curve.

for PGE_2 and 14.82 nM for $PGF_{2\alpha}$, an 18.5-fold higher affinity for PGE_2, indicating that PGE_2 actually has a greater affinity for ^{3}H-$PGF_{2\alpha}$ binding sites. Iris and ciliary body membranes were not examined for competition studies with $PGF_{2\alpha}$ because they did not significantly bind this ligand.

Selectivity Study of Sphincter $PGF_{2\alpha}$ Binding Sites. A limited study was performed to determine whether $PGF_{2\alpha}$ (Table 2) was binding to EP or FP binding sites (Fig. 3). No difference was observed in the ability of 4–4000 nM of the highly selective FP agonist, 17-phenyl trinor $PGF_{2\alpha}$ (Table 2) versus the less selective $PGF_{2\alpha}$ (Table 2) to displace 4 nM ^{3}H-$PGF_{2\alpha}$ or ^{3}H-PGE_2 when compared to the displacement by 4–400 nM of $PGF_{2\alpha}$. In addition, 4–400 nM PGE_2 was more effective than either $PGF_{2\alpha}$ and 17-phenyl trinor $PGF_{2\alpha}$ at displacing either labeled ligand.

Displacement of ^{3}H-PGE_2 or ^{3}H-$PGF_{2\alpha}$ by EP, FP, DP and IP Agonists and a DP Antagonist in the Sphincter Muscle. The selectivity of ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ binding sites was examined by testing their displacement by EP, FP, DP, and IP selective agonists as well as a DP selective antagonist. A single concentration (400 nM) of each agonist, equivalent to the dose of PGE_2 that produces 80% displacement of labeled PGE_2 and $PGF_{2\alpha}$ (see Fig. 3) was used. The selectivity of these agonists and antagonists is detailed in Table 2.

Displacement of ^{3}H-PGE_2

Figure 4 illustrates the ability of IP, EP, FP, and DP selective agonists, and a DP selective antagonist to displace 4 nM ^{3}H-PGE_2 from its specific binding sites in the bovine sphincter membrane preparation. The IP agonist Iloprost (Table 2) marginally displaced ^{3}H-PGE_2, indicating that the radioligand was not bound to IP sites. The DP agonist PGD_2 and the DP selective antagonist BWA868C[28] displaced some of the bound radioligand (Table 2), although significantly less than that displaced by unlabeled PGE_2. The highly selective antagonist, BWA868C, displaced even less radioligand than PGD_2 indicating that DP receptors are probably not involved. $PGF_{2\alpha}$ and 17-phenyl trinor $PGF_{2\alpha}$ displaced approximately equivalent amounts of the radioligand, although the FP selective 17-phenyl trinor $PGF_{2\alpha}$ displaced less ^{3}H-PGE_2 than did $PGF_{2\alpha}$. This lack of selectivity in displacement suggests that ^{3}H-PGE_2 is not bound to FP sites.

The nonselective EP agonist PGE_2 (Table 2) displaced more ^{3}H-PGE_2 than any other agonist or antagonist. The EP_2/EP_3 (11-deoxy PGE_1) and EP_1 (17-phenyl trinor PGE_2) selective agonists (Table 2) were nearly as effective as unlabeled PGE_2 and significantly

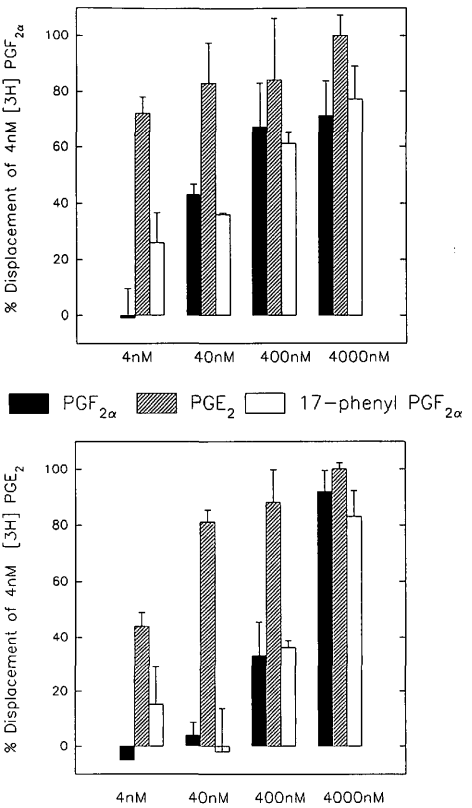

FIGURE 3. Comparison of the selectivity of $PGF_{2\alpha}$, 17-phenyl trinor $PGF_{2\alpha}$ and PGE_2 for displacement of either 4 nM ^{3}H-$PGF_{2\alpha}$ (*upper panel*) or ^{3}H-PGE_2 (*lower panel*) specific binding to bovine sphincter muscle membranes. Incubation conditions were the same as those in Fig. 1 (n = 3 for each ligand).

TABLE 2. Prostanoid Receptor Agonists and Antagonists Used to Displace Radiolabeled PGE_2 or $PGF_{2\alpha}$ From the Sphincter Membrane Preparation

Compound	*Selectivity*	*Reference*
Agonists (all 400 nM)		31, 32, 36
PGE_2	EP	28, 29, 33
17-Phenyl trinor PGE_2	EP1	27–30, 32, 39
11-Deoxy PGE_1	EP2 > EP3	14, 31
$PGF_{2\alpha}$	FP > EP	29, 32
17-Phenyl trinor $PGF_{2\alpha}$	FP	29, 30
PGD_2	DP > TP	32, 33
Iloprost	IP > EP1	32, 36
Antagonist (4 μM)		
BWA868C	DP	28

The relative selectivity of each compound is indicated.

more effective than $PGF_{2\alpha}$ or 17-phenyl $PGF_{2\alpha}$ at displacing ^{3}H-PGE_2. This evidence suggests that the radioligand ^{3}H-PGE_2 is highly selective for EP binding sites in bovine sphincter. Because the K_d value for PGE_2 in sphincter muscle is 3.1 nM (Table 1), the concentration of EP selective agonists used (400 nM) were not chosen to demonstrate whether EP_2 or EP_1 sites predominate but to demonstrate ^{3}H-PGE_2 selectivity for EP versus non-EP binding sites.

Displacement of 3H-PGF_2

Figure 5 illustrates the ability of IP, EP, FP, and DP selective agonists and a DP selective antagonist to displace 4 nM ^{3}H-$PGF_{2\alpha}$ from its binding sites in the bovine sphincter membrane preparation. Iloprost was unable to displace any of the ^{3}H-$PGF_{2\alpha}$, indicating that it was not bound to IP sites. The DP agonist PGD_2 and the selective antagonist BWA868C did displace some of the bound radioligand, although significantly less than that displaced by unlabeled $PGF_{2\alpha}$. The highly selective DP antagonist displaced even less radioligand than PGD_2, indicating that DP receptors may be only marginally involved.

Unlabeled $PGF_{2\alpha}$ and 17-phenyl trinor $PGF_{2\alpha}$ displaced equivalent amounts of the ^{3}H-$PGF_{2\alpha}$. The EP subtype selective agonists 11-deoxy PGE_1 and 17-phenyl trinor PGE_2 were nearly as effective as $PGF_{2\alpha}$ at displacing radioligand. The nonselective EP agonist PGE_2, displaced more radioligand than any other agonist or antagonist. The lack of selectivity in displacement of ^{3}H-$PGF_{2\alpha}$ combined with the effectiveness of EP selective agonist suggests that, ^{3}H-$PGF_{2\alpha}$ does not bind selectively to FP sites but nonselectively to other, predominantly EP sites.

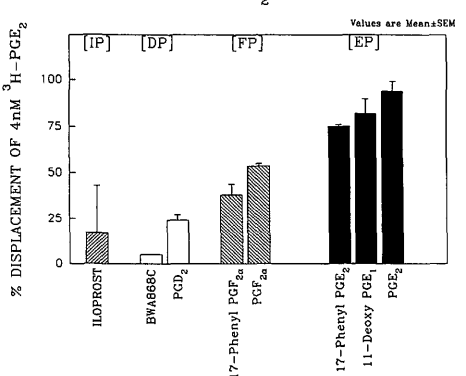

FIGURE 4. Comparison of the ability of IP, EP, FP, and DP selective agonists and antagonist to displace 4 nM ^{3}H-PGE_2 from its specific binding sites in the bovine sphincter membrane preparation. Concentration of all agonists are 400 nM. The concentration of the antagonist BWA868C is 4 μM. Incubation conditions were the same as those in Fig. 1 except the membranes receiving antagonist were preincubated for 10 minutes with antagonist before addition of radioligand (n = 3). Values are the mean ± SEM.

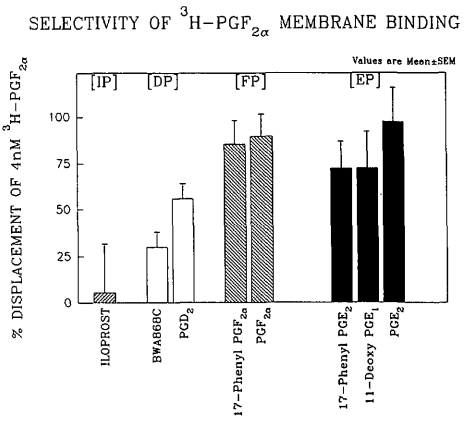

FIGURE 5. Comparison of the ability of IP, EP, FP, and DP selective agonists and antagonist to displace 4 nM ^{3}H-$PGF_{2\alpha}$ from its specific binding sites in the bovine sphincter membrane preparation. Concentration of all agonists are 400 nM. The concentration of the antagonist BWA868C is 4 μM. Incubation conditions were the same as those in Fig. 1 except the membranes receiving antagonist were preincubated for 10 min with antagonist before addition of radioligand (n = 3). Values are mean ± SEM.

DISCUSSION

In the current study we have demonstrated the regional distribution and binding characteristics of ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ binding sites in membrane preparations of separated bovine sphincter muscle, iris, and ciliary body. Our previously reported K_d and B_{max} values for PGE_2 in the whole iris ciliary body were 13 nM and 162 fmoles/mg protein, respectively.[11] Data from the present study indicate a lower K_d (3.1 nM) and a higher B_{max} (842 fmoles bound/mg protein) in the isolated sphincter muscle, whereas iris and ciliary body K_d values are higher and B_{max} values are lower than whole tissue values. Iris sphincter receptor density is fivefold higher than iris and tenfold higher than ciliary body.

For comparison, the K_d for PGE_2 in bovine luteal cells is 2.4 nM.[34] In baboon and rabbit uterine membranes,[35] the K_d values for PGE_2 are in the range of 3.3–13 nM; similar to the 3.1–28 nM range we found in separated ocular tissues. In rat kidney,[36] the K_d and B_{max} values for PGE_2 and $PGF_{2\alpha}$ were 9.37 and 9.17 nM, and 2150 and 440 fM bound/mg protein, respectively. The regional distribution of ^{3}H-PGE_2 binding sites was examined in the human kidney.[37] K_d values

for PGE_2 ranged from 3.7 nM in the cortex to 6.2 nM in the outer medulla; and B_{max} values ranged from 143 fM bound/mg protein in the cortex to 335 fM bound/mg protein in the outer medulla. The affinity of PGE_2 for its binding sites does not appear to vary greatly between species or tissues. However, the relatively low B_{max} values for these binding sites and the lipophilic character of the agonist used served to increase the errors in measurement and rendered collection of direct binding data more difficult.

Another focus of the current study was to examine the selectivity of EP and FP binding sites in bovine sphincter muscle. In human kidney[37] PGE_2 is 51 times more effective than $PGF_{2\alpha}$ at displacement of specifically bound ^{3}H-PGE_2; the rank order of potency of selected prostanoid agonists is $PGE_2 \rangle PGF_{2\alpha} \rangle PGI_2 \equiv PGD_2$. Our data using 17-phenyl $PGF_{2\alpha}$ demonstrated that sites selective for FP verses EP agonists may not exist in the iris sphincter (Fig. 3). In addition, the data in Figure 4 indicate the same order of potency for prostanoid agonist displacement of ^{3}H-PGE_2 in our bovine sphincter membrane preparation as reported for human kidney EP sites. The rank order of potency of prostanoid agonist displacement of ^{3}H-$PGF_{2\alpha}$ (Fig. 5) from bovine sphincter muscle was $PGE_2 \equiv PGF_{2\alpha} \rangle PGD_2 \rangle PGI_2$. $PGF_{2\alpha}$ has been reported to compete with PGD_2 for DP binding sites[30] and the data suggest (Fig. 5) the possibility of a small contribution of ^{3}H-$PGF_{2\alpha}$ bound to DP sites. Overall, these results demonstrate that ^{3}H-$PGF_{2\alpha}$ was predominately bound to EP rather than FP sites and raises the possibility that the bovine iris does not contain a significant number of FP receptor sites.

One may make correlations between these binding data and the functional response of bovine iris ciliary body to prostaglandins. PGE_2 and $PGF_{2\alpha}$ are potent agonists for contractile responses in isolated iris sphincter muscle.[21-23] Dong et al[21] reported that the equimolar ratio for contraction of bovine sphincter by $PGF_{2\alpha}$ versus PGE_2 to be 53:1. The addition of the TP receptor antagonists EP045 and EP092 had little effect on these molar ratios. The potent IP receptor agonist ZK96480 exhibited weak contractile activity, suggesting that the bovine sphincter contractile response to Iloprost is caused by partial agonist activity at EP sites. Suzuki and Kobayashi[22] demonstrated that contraction of bovine iris occurred as a direct result of prostaglandin binding to muscle rather than to nerve; indicating the presence of PG receptor sites associated with intraocular muscle membranes. These same authors examined the contractile response of isolated bovine iris sphincter segments to PGE_1, PGE_2, and $PGF_{2\alpha}$. Dose-response curves for PGE_2 and PGE_1 exposure began at 1 nM and are maximal below 10 μM. The response to $PGF_{2\alpha}$ begins at 100 nM and by 10 μM reaches only 10% of the maximal PGE_2 contractile response. Although the bovine iris sphincter responded to both PGE_2 and $PGF_{2\alpha}$, PGE_2 was two orders of magnitude more effective than $PGF_{2\alpha}$; data that correspond well to binding data from our current competition studies (Fig. 1). Data from the current study indicating that the K_d for PGE_2 in bovine sphincter is 3.1 nM correlate well to a functional response curve that begins at 1nM.

Yoshitomi and Ito[19] and van Alphen et al[20] demonstrated that dog and cat iris sphincter responses to PGE_2 and $PGF_{2\alpha}$ were opposite those of bovine iris sphincter, and were 100-fold more sensitive to $PGF_{2\alpha}$ than to PGE_2. In addition, contractile response curves to $PGF_{2\alpha}$ begin at less than 0.1 nM, suggesting a very low K_d value for $PGF_{2\alpha}$ in these tissues. Bovine iris sphincter muscle responsiveness to $PGF_{2\alpha}$-ester exposure lies between the more responsive cat and dog and the less responsive rabbit, pig, guinea pig, and human.[38] Dog dilator muscle contracts to $PGF_{2\alpha}$ exposure; with only a marginal response to PGE_2 and PGE_1.[19] We found no appreciable specific $PGF_{2\alpha}$ binding to the membrane preparation derived from bovine iris containing dilator muscle fibers. Suzuki and Kobayashi[22] report that the bovine iris dilator muscle responds to $PGF_{2\alpha}$ but not PGE_2 or PGE_1; however, in their study, 1 μM $PGF_{2\alpha}$ was required to produced a small change in the electrically evoked contraction of iris dilator muscle and had no effect on ciliary muscle. This may help explain why only marginal $PGF_{2\alpha}$ binding to these tissues was observed in our study. Alternately, there may be no FP receptors present in these tissues.

Woodward et al, 1989[29] demonstrated that $PGF_{2\alpha}$ induced ocular hypertension in cats was negatively correlated to FP receptor stimulation and positively correlated to iris sphincter contraction. They conclude that $PGF_{2\alpha}$ may alter intraocular pressure by binding to a prostanoid receptor other that those of the FP or TP classification. Their study demonstrates that prostanoids introduced into the anterior chamber may not exert a single discrete physiological response in this tissue. Our finding of a lack of measurable FP receptor sites in bovine iris and ciliary body may rule out a functional role for these receptors. We cannot directly correlate the EP binding sites we identified with functional receptors responsible for muscle contraction. However, it is generally accepted that IP_3 release and the subsequent intracellular Ca^{++} elevation are closely involved in sphincter muscle contraction.[38] In the cat and dog iris sphincter $PGF_{2\alpha}$ apparently binds to an FP receptor[12] stimulating the release of IP_3[38] with the subsequent contraction of the muscle. If we postulate a similar mechanism for the bovine iris sphincter $PGF_{2\alpha}$ or PGE_2 would bind to an EP receptor to contract the muscle. EP_2 and EP_3 receptor subtype activation results in a stimulation or inhibition,

respectively, of cyclic adenosine monophosphate release. The release of IP_3 after EP_1 receptor subtype stimulation may be responsible for sphincter contraction. Yousafzai[38] suggested that the predominance of the IP_3 second messenger pathway in the cat and dog iris sphincter explains a high sensitivity to $PGF_{2\alpha}$. In rabbits, humans, and other species they suggest an inverse relationship between the action of the IP_3 and cyclic adenosine monophosphate second messenger pathways on contraction and relaxation, which results in a sphincter muscle that is nonresponsive to both $PGF_{2\alpha}$ and PGE_2. Perhaps bovine contractile responses are mediated by an EP_1 not an FP receptor. The result is a sphincter muscle that is responsive to PGE_2 but exhibits an intermediate sensitivity to $PGF_{2\alpha}$ that falls between the more sensitive dog and cat, and the less sensitive human, rabbit, and guinea pig sphincter muscle.

Our current study has determined the localization and kinetic parameters of PGE_2 and $PGF_{2\alpha}$ binding sites in the bovine eye; however, these findings point out how little is understood about the outcome of prostanoid interaction with their receptors in ocular tissue.

Key Words

PGE_2, $PGF_{2\alpha}$, receptor, binding sites, bovine, sphincter, iris-ciliary body

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