Prostaglandin $F_{2\alpha}$ Effects on Intraocular Pressure Negatively Correlate with FP-Receptor Stimulation


According to the current working classification for prostanoid receptors, the prostaglandin $F_{2\alpha}$-sensitive receptor (FP-receptor) may be identified by comparing the rank order of activity of prostaglandin $F_{2\alpha}$ (PGF$_{2\alpha}$) and its analogues. In order to further understand the pharmacology of PGF$_{2\alpha}$-induced ocular hypotension, the intraocular pressure response to PGF$_{2\alpha}$ and selected analogues was compared with their rank order of activity in typical FP-receptor preparations such as contraction of the cat iris sphincter and affinity for corporal luteal membrane binding sites. The rank order of potency for decreasing intraocular pressure was as follows: PGF$_{2\alpha}$ > PGF$_{1\alpha}$ > 16-phenoxytetranor PGF$_{2\alpha}$ > 17-phenyltrinor PGF$_{2\alpha}$ = fluprostenol (inactive). For cat iris sphincter contraction, the rank order of potency appears to be fluprostenol = 17-phenyltrinor PGF$_{2\alpha}$ > 16-phenoxytetranor PGF$_{2\alpha}$ = PGF$_{2\alpha}$ > PGF$_{1\alpha}$. The rank order of potency for PGF$_{2\alpha}$ analogues in decreasing intraocular pressure appears to negatively correlate with the rank order for cat iris sphincter contraction and literature values for corporal luteal membrane binding. It is concluded that the ocular hypotensive effect of PGF$_{2\alpha}$ is not mediated by the FP-receptor. Invest Ophthalmol Vis Sci 30:1838-1842, 1989

Prostaglandin $F_{2\alpha}$ (PGF$_{2\alpha}$) has been shown to lower intraocular pressure in a variety of laboratory animals$^{1-4}$ and man,$^5$ and the prospect of a PGF$_{2\alpha}$-like agent as an antiglaucoma therapy is a current topic of interest. In addition to its ocular hypotensive action, PGF$_{2\alpha}$ has a number of other biological activities, some of which have previously attracted considerable attention. The role of PGF$_{2\alpha}$ in reproduction and fertility was an early focus of attention, but lack of metabolic stability and tissue selectivity precluded its widespread use for regulating reproductive processes. This problem provided an impetus for medicinal chemistry studies. Introduction of bulky substituents in the vicinity of the 15-OH group of PGF$_{2\alpha}$ provided longer-acting derivatives by prevention of rapid metabolism by 15-OH prostaglandin dehydrogenase (15-OH PGDH) and $\omega$-oxidation. Moreover, these structural modifications frequently increased affinity and selectivity for PGF$_{2\alpha}$-sensitive receptors and increased luteolytic potency.$^6-9$ The degree of selectivity and potency of certain synthetic PGF$_{2\alpha}$ derivatives, such as fluprostenol, has been sufficient to provide substantial evidence for subclassification of prostanoid receptors, with the specific prostanoid receptor for PGF$_{2\alpha}$ designated as the FP-receptor by the current working classification.$^9,10$ The dog and cat iris sphincter muscles and corporal luteal radioligand binding assays have proved particularly useful preparations for studying the FP-receptor.$^9,10$

The purpose of the studies described herein was to investigate the pharmacology of PGF$_{2\alpha}$-induced ocular hypotension by comparing the relative activity of prostaglandin $F_{1\alpha}$ (PGF$_{1\alpha}$), PGF$_{2\alpha}$, and selective FP-receptor agonists as typically represented by fluprostenol.$^9,11$ Comparison of ocular hypotension and pupil diameter in the cat provided a particularly useful model since contraction of the cat iris sphincter is considered as an FP-receptor-mediated response.$^9,10$ Furthermore, differential miotic and ocular hypotensive responses to natural prostanoids have been previously noted in cats.$^1,3$

Materials and Methods

Intraocular pressure studies were performed in two laboratory animal species: (1) New Zealand, albino/Dutch-belted cross-bred rabbits of either sex and weighing 1.5-2.5 kg; and (2) domestic cats of either sex weighing 2.5-4 kg. The rabbits had not previously received topical drugs of any kind; the cats had not received topical drugs for a period of at least 1 month before each study. The cats had been trained for a minimum of 6 months so that tonometry could be performed without the use of restraining devices. Intraocular pressure was measured with a pneumonometer (Digilab) calibrated against the eyes of anes-
Fig. 1. Effect of graded doses of PGF$_2$\alpha, PGF$_2$\beta, 16-phenoxytetranor PGF$_2$\alpha, 17-phenyltrinor PGF$_2$\beta, and fluprostenol on rabbit intraocular pressure. Eyes that received PGF$_2$\alpha (a) 0.01%, (b) 0.1%, (c) 1%; PGF$_2$\beta (d) 0.01%, (e) 0.1%, (f) 1%; 16-phenoxytetranor PGF$_2$\alpha (g) 0.1%, (h) 1%; 17-phenyltrinor PGF$_2$\beta (i) 0.1%, (j) 1%; fluprostenol (k) 0.1%, (l) 1% are represented by (•). Contralateral, control eyes are represented by (○). Points are mean values ± SEM. *P < 0.05 and **P < 0.01 represent significant decreases in intraocular pressure relative to baseline (0 hr); †P < 0.05 and ‡P < 0.01 represent significant increases in intraocular pressure relative to baseline (0 hr). n = 8.

The effect of topically applied PGF$_2$\alpha and its derivatives on pupil diameter in cats was determined as the shortest median-lateral pupil measurement obtained with a millimeter scale gauge. The light level was ad-
The in vivo ocular effects of topically administered PGF₂α and its derivatives were evaluated by applying 25 μl of drug solution to one eye and 25 μl of vehicle to the contralateral eye as a control. PGF₁α, PGF₂α, 16-phenoxytetranor PGF₂α, 17-phenyltrinor PGF₂α, and fluprostenol were purchased from Cayman Chemical (Kalamazoo, MI), and 16 m-trifluoromethyl phenoxy-17,18,19,20-tetranor prostaglandin F₂α (fluprostenol) was a gift from Cooper (Berkhamsted, UK). These prostanoids were dissolved in 2% Na₂CO₃ and neutralized with HCl. Intraocular pressure and, in the case of cats, pupil diameter, were measured at 30 min and immediately before topical prostanoid administration and at 30, 60, 120, 180, 240 and 360 min thereafter. Statistical analysis employed student paired t-test. All experiments were performed according to the ARVO Resolution on the Use of Animals in Research.

### Results

The effects of PGF₂α, PGF₁α, 16-phenoxytetranor PGF₂α, 17-phenyltrinor PGF₂α, and fluprostenol on rabbit intraocular pressure are compared in Figure 1. PGF₂α produced a dose-dependent decrease in intraocular pressure over the 0.01%–1% concentration range (Fig. 1a–c). 0.1% and 1% concentrations of PGF₂α also caused an increase in intraocular pressure that was dose-dependent and rapid in onset (Fig. 1b, c). A similar profile of activity was apparent for PGF₁α, although it appeared slightly less potent than PGF₂α. A 0.01% concentration of PGF₁α was virtually inactive (Fig. 1d), whereas a 0.1% concentration produced a distinct ocular hypotensive response (Fig. 1e). At a 1% concentration, PGF₁α also caused a marked increase in intraocular pressure followed by the ocular hypotensive response (Fig. 1f). At both 0.1% and 1% concentrations, 16-phenoxytetranor PGF₂α produced only a small decrease in intraocular pressure that achieved a statistically significant level at only two timepoints (Fig. 1g, h). No decrease in intraocular pressure was apparent for 17-phenyltrinor PGF₂α at either the 0.1% or 1% concentration (Fig. 1i, j). The selective FP-receptor agonist fluprostenol did not alter intraocular pressure at any dose employed (Fig. 1k, l). In several instances, intraocular pressure values increased in the latter part of the study. This appears, however, to be unrelated to drug effects and is a common feature of such experiments in this rabbit strain.

The effects of prostanoids on cat intraocular pressure and pupil diameter were compared at a single (0.1%) concentration. The distinct decrease in intraocular pressure produced by PGF₂α did not occur in to provide a strip approximately 3 mm wide and 15–20 mm long. The iris sphincter preparation was mounted vertically under 150 mg tension in a jacketed 5 ml organ bath. The organ bath contained Tyrode solution of the following composition (mM): NaCl (137), KCl (2.7), MgSO₄·6H₂O (1.0), CaCl₂·2H₂O (1.9), NaHCO₃ (11.9), KH₂PO₄ (0.5), and dextrose (5.6). The solution was aerated and maintained at 37.5°C. The effect of prostanoids on muscle tension was measured isometrically with a force displacement transducer (Grass FT-03) and recorded on a Grass polygraph (Model 7D). A 60 min stabilization period was allowed for each tissue. Prostanoids were added cumulatively to the organ bath and following wash-out at least 60 min was allowed for recovery of the preparation.

All experiments were performed according to the ARVO Resolution on the Use of Animals in Research.
response to 16-phenoxytetranor PGF$_{2\alpha}$, 17-phenyltrinor PGF$_{2\alpha}$, and fluprostrenol (Fig. 2a–d). A transient hypertensive response to 16-phenoxytetranor PGF$_{2\alpha}$ was also observed (Fig. 2b). The effect of PGF$_{2\alpha}$ and its analogues on pupil diameter is depicted in Figure 3. Fluprostrenol (Fig. 3d) and 17-phenyltrinor PGF$_{2\alpha}$ (Fig. 3c) behaved in a virtually identical manner by producing a marked and prolonged miotic response similar to that evoked by PGF$_{2\alpha}$ (Fig. 3a). Figure 3b shows that 16-phenoxytetranor PGF$_{2\alpha}$ also produced marked miosis, but this was relatively transient compared to the response evoked by PGF$_{2\alpha}$, 17-phenyltrinor PGF$_{2\alpha}$, and fluprostrenol.

The effects of PGF$_{2\alpha}$, 16-phenoxytetranor PGF$_{2\alpha}$, and 17-phenyltrinor PGF$_{2\alpha}$ on the isolated cat iris sphincter muscle are shown by Figure 4. All three prostanoids potently contracted the cat iris sphincter. The dose–response curves for PGF$_{2\alpha}$ and 16-phenoxytetranor PGF$_{2\alpha}$ were virtually identical, whereas 17-phenyltrinor PGF$_{2\alpha}$ appeared to be approximately one-half of a log$_{10}$ unit more potent.

**Discussion**

These studies indicate that the ocular hypotensive response to PGF$_{2\alpha}$ cannot be attributed to FP-receptor stimulation. Although PGF$_{2\alpha}$ produced pronounced decreases in intraocular pressure, the potent and selective FP-receptor agonist fluprostrenol was inactive. Additional evidence to support the postulate that PGF$_{2\alpha}$-induced ocular hypotension does not involve the FP-receptor was obtained from comparing the actions of additional PGF$_{2\alpha}$ analogues. 17-Phenyltrinor PGF$_{2\alpha}$ and 16-phenoxytetranor PGF$_{2\alpha}$ were found to have little or no effect on intraocular pressure despite their previously reported high affinity for the corporal luteal PGF$_{2\alpha}$-receptor and their potent myotropic activity in a further FP-receptor preparation, namely the cat iris sphincter. PGF$_{1\alpha}$ was also selected for study since this has relatively low affinity for the corporal luteal PGF$_{2\alpha}$ receptor and does not potently contract the cat iris sphincter. Although less active than PGF$_{2\alpha}$, PGF$_{1\alpha}$ caused pronounced decreases in intraocular pressure at both 0.1% and 1% doses. The rank order of potency for decreasing intraocular pressure may be summarized as follows: (PGF$_{2\alpha}$) > PGF$_{1\alpha}$ > 16-phenoxytetranor PGF$_{2\alpha}$ > 17-phenyltrinor PGF$_{2\alpha}$ = fluprostrenol (inactive). This, essentially, correlates negatively with the rank order of potency for these PGF$_{2\alpha}$ analogues in FP-receptor preparations. Thus, combining the present data with previously reported values, the rank order of potency for contraction of the cat iris sphincter appears to be fluprostrenol = 17-phenyltrinor PGF$_{2\alpha}$ > 16-phenoxytetranor PGF$_{2\alpha}$ (>PGF$_{2\alpha}$). A very similar rank order of affinity for corporal luteal PGF$_{2\alpha}$ binding sites has been reported.

![Figure 3](http://example.com/fig3.png)

**Figure 3.** Effect of 0.1% (a) PGF$_{2\alpha}$, (b) 16-phenoxytetranor PGF$_{2\alpha}$, (c) 17-phenyltrinor PGF$_{2\alpha}$, (d) fluprostrenol on cat pupil diameter. Points are mean values ± SEM. *P < 0.05 and **P < 0.01 represent significant changes from baseline (0 hr) pupil diameter. n = (a) 6, (b) 10, (c) 6, (d) 6.

![Figure 4](http://example.com/fig4.png)

**Figure 4.** Log (M) concentration–response curves for PGF$_{2\alpha}$ (●), 16-phenoxytetranor PGF$_{2\alpha}$ (▲), and 17-phenyltrinor PGF$_{2\alpha}$ (■) on the isolated cat iris sphincter muscle. Points are mean values for the % maximal contractile response for each prostanoid ± SEM. n = 6 for PGF$_{2\alpha}$ and n = 5 for 16-phenoxytetranor PGF$_{2\alpha}$ and 17-phenyltrinor PGF$_{2\alpha}$. 

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Although these studies indicate that PGF\textsubscript{2α} does not lower intraocular pressure by stimulating the FP-receptor, they do not reveal which prostanoid receptor is involved in mediating this effect. PGF\textsubscript{2α} can behave as a thromboxane A\textsubscript{2} (TxA\textsubscript{2}) mimic as exemplified by pharmacological studies in respiratory smooth muscle preparations. Thus, both TxA\textsubscript{2} mimetics and PGF\textsubscript{2α} cause contraction of respiratory smooth muscle preparations and these responses are antagonized by TxA\textsubscript{2} antagonists\textsuperscript{13-15}. TxA\textsubscript{2} mimetics, however, have very little effect on intraocular pressure\textsuperscript{3,16}, and it follows that the TxA\textsubscript{2}-sensitive (TP) receptor\textsuperscript{9,10} is not involved in PGF\textsubscript{2α}-induced ocular hypertension. Considering the current prostanoid receptor classification\textsuperscript{9,10} and the absence of FP- or TP-receptor participation, involvement of the other known prostanoid receptor subtypes in PGF\textsubscript{2α}-induced ocular hypotension cannot be dismissed. However, in view of the high potency of PGF\textsubscript{2α} in lowering intraocular pressure, the involvement of a novel PGF\textsubscript{2α}-sensitive receptor subtype is also a distinct possibility.

The lack of ocular hypertensive activity associated with FP-receptor agonists administered to rabbits also deserves mention in that, to date, ocular hypertensive and hypotensive responses to PGF\textsubscript{2α} have been pharmacologically inseparable in this species. Curiously, 16-phenoxytetranor PGF\textsubscript{2α} caused an ocular hypertensive response in cats, the pharmacological basis of which remains to be determined. This unexpected finding suggests that fluprostenol and 17-phenyltrinor PGF\textsubscript{2α} are more suitable agents for studying ocular FP-receptors.

Key words: prostaglandin F\textsubscript{2α}, intraocular pressure, cat iris

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References