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EFFECTS OF PROSTAGLANDINS AND THROMBOXANE ANALOGUES ON BULLOCK AND DOG IRIS SPHINCTER PREPARATIONS

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1 The bullock iris sphincter was contracted by low concentrations of prostaglandin E_2 (PGE_2), 16,16-dimethyl PGE_2 and 17,18,19,20-tetranor-16-*p*-chlorophenoxy PGE_2. Other compounds with thromboxane-like actions, for example 11,9-epoxymethano PGH_2, were also potent spasmogens. ZK 36374, a stable carbacyclin, was a partial agonist on the PGE-sensitive system of this tissue.

2 The thromboxane antagonist, EP 045, had little effect on the action of PGE_2 and 16,16-dimethyl PGE_2 on the bullock iris.

3 The dog iris sphincter was sensitive to $PGF_{2\alpha}$ but not to PGE_2 and 11,9-epoxymethano PGH_2.

4 16,16-Dimethyl PGE_2 had very low activity on the dog iris in contrast to its high activity on the bullock iris. The reverse was found with the 17,18,19,20-tetranor-16-*m*-trifluoromethylphenoxy analogue of $PGF_{2\alpha}$ (ICI 81008). This indicates a considerable selectivity of action of the two analogues.

5 The results are discussed in relation to the existing knowledge of prostanoid receptors.

Introduction

Interest in the actions of prostaglandins on the eye stems from the identification of E- and F-type prostaglandins as components of irin from sheep (Änggård & Samuelsson, 1964), rabbit and cat (Ambache, Brummer, Rose & Whiting, 1966) and bullock (Posner, 1970) irises. Dorp, Jouvenaz & Struijk (1967) also showed that pig iris could convert all *cis*-8,11,14-eicosatrienoic acid into prostaglandins E_1 and $F_{1\alpha}$.

Several workers have made pharmacological comparisons of prostaglandins on isolated iris smooth muscle. The dog and cat sphincter muscles are of particular interest since prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) produces a contractile effect at very low concentrations and is much more active than PGE_2 and stable thromboxane-like agents such as 11,9-epoxymethano PGH_2 (Alphen & Angel, 1975; Coleman, Humphrey, Kennedy, Levy & Lumley, 1981). In contrast the bullock iris sphincter is more responsive to PGE_2 than $PGF_{2\alpha}$ (Posner, 1973). We decided to characterize further the PGE-sensitive system in the bovine iris sphincter, with a view to comparing it with other smooth muscle preparations responsive to PGE_2 and related analogues.

Methods

Bullock eyes were enucleated just after slaughter, placed in ice-cold sugar-salt solution (KCl 0.35, NaCl 6.95, $CaCl_2$ 0.24, $MgSO_4.7H_2O$ 0.29, KH_2PO_4 0.16, $NaHCO_3$ 1.25, dextrose 1.0, sucrose 17 and EDTA 0.01 g/l) (Crawford, Alphen, Cook & Lands, 1978), and used within 12 h. Two strips of the sphincter muscle, about 10 mm long and 2.5 mm wide, were dissected from the upper and lower margins of the iris. Each preparation was mounted under 40–60 mg tension in 10 ml of the bathing solution containing indomethacin (10^{-6} M), aerated with 95% O_2/5% CO_2 and kept at 37°C. The effect of drugs on muscle tension was measured isometrically with a Grass force displacement transducer (FT 03C) and recorded on a Grass Polygraph (Model 7C). Each preparation was allowed 1 h to stabilize and drugs were added cumulatively to the organ bath (contact time for each concentration being 3–5 min in most cases). Following wash-out the preparation was allowed 30–60 min to recover before addition of another compound.

Dog iris sphincter preparations were obtained by similar procedures from eyes removed during pentobarbitone anaesthesia.

Prostaglandins were dissolved in 0.9% w/v NaCl solution (saline) with addition of small amounts of sodium bicarbonate when necessary. PGI_2 sodium salt (Schering) was dissolved in 0.05 M Tris HCl, pH 9.0, to give a 1 mg/ml solution. Serial dilutions of the PGI_2 stock were made with saline, kept on ice, and

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used for one cumulative dose sequence only. It should be noted that a bath concentration of Tris HCl in excess of 0.2 μM will potentiate the contractile action of PGI_2 and ZK 36374.

Results

Bullock iris sphincter muscle

Relative potencies of prostaglandins As a routine procedure the bathing fluid contained indomethacin (10^{-6} M); this ensured a stable basal tension over a period of at least 5 h. PGE_2 was used as the standard agonist and doses were added cumulatively to the organ bath. Preparations were reproducibly responsive to PGE_2. Threshold responses (tension change of about 20 mg) were seen with concentrations of 0.2–0.8 ng/ml and 50% maximum responses (150–400 mg) with 1–6 ng/ml. Representative log concentration-effect curves for several of the analogues (see Figure 1 for chemical structures) are shown in Figure 2. Log concentration-effect curves for all the analogues listed in Table 1 were approximately parallel to that of PGE_2. Equipotent molar ratios (Table 1) were derived from the molar concentration of PGE_2 giving a response 50% of its own maximum and the molar concentration of the test compound producing an equivalent response.

On eight preparations the 11,9-epoxymethano analogue of PGH_2 (U-46619) showed similar contractile potency to PGE_2 but its log concentration-

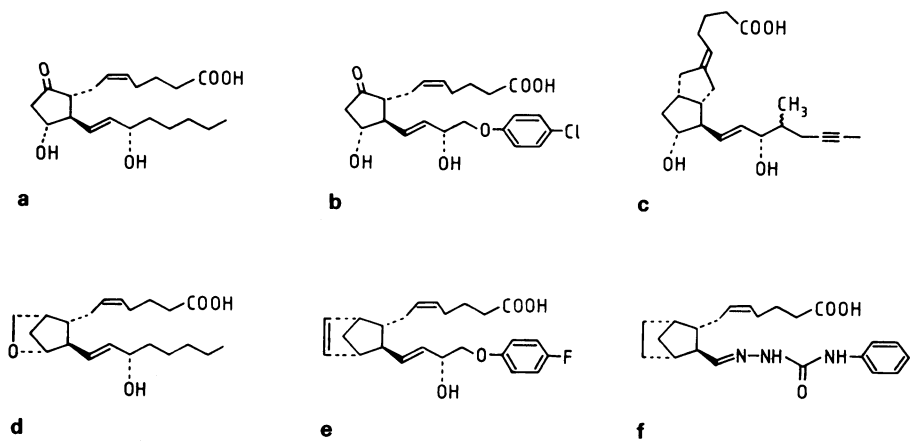

Figure 1 Structures of (a) PGE_2, (b) ICI 80205, (c) ZK 36374, (d) 11, 9-epoxymethano PGH_2, (e) EP 011 and (f) EP 045.

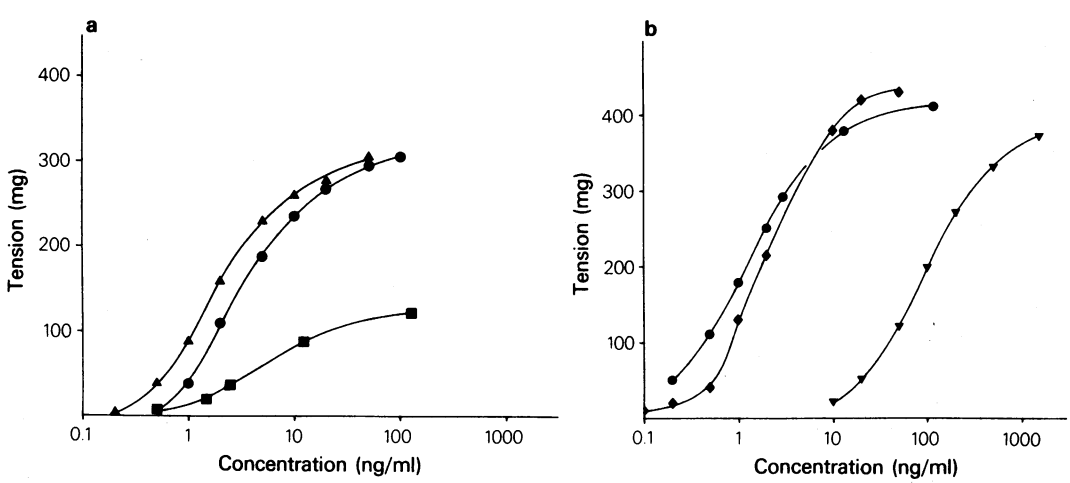

Figure 2 Bullock iris sphincter muscle preparations: cumulative concentration-response relationships for (a) PGE_2 (●), 16,16-dimethyl PGE_2 (▲) and ZK 36374 (■); (b) PGE_2 (●), 11,9-epoxymethano PGH_2 (◆) and $PGF_{2\alpha}$ (▼).

Table 1 Potencies of prostaglandin analogues on the bullock isolated iris sphincter preparation

Compound	*Equipotent molar ratio* ($PGE_2 = 1.0$)
(±)-17,18,19,20-tetranor-16-*p*-chlorophenoxy PGE_2 (ICI 80205)	0.12, 0.17, 0.17
16, 16-dimethyl PGE_2	0.50 ± 0.09 ($n = 7$)
PGE_1	5.3, 9.3
(±)-11-deoxy PGE_1	37, 47
PGD_2	480, 690, 2000
$PGF_{2\alpha}$	19, 56, 63, 86
(±)-17,18,29,20-tetranor-16-*m*-trifluoromethylphenoxy $PGF_{2\alpha}$ (ICI 81008)	(640), (650), 1000
PGI_2	40, 100, 108, 170, 180

Individual values are the result of a comparison with PGE_2 on a single preparation. With ICI 81008, the full concentration-response relationship was not established in two experiments and the corresponding equipotent molar ratios in parentheses relate to responses at the 20% maximum response level.

effect curve was always slightly steeper and its maximum response greater (5–25%). At the 50% maximum response level for PGE_2, 11,9-epoxymethano PGH_2 was 1.25 (range 0.50–2.7, $n = 8$) times less active than PGE_2.

Addition of PGE_1, PGE_2 or 11,9-epoxymethano PGH_2 to a preparation partially contracted with carbachol resulted in a further increase in tension: there was no evidence for a prostaglandin-mediated relaxant effect on this preparation. Adrenaline (200 ng/ml) completely inhibited the contractile action of carbachol.

Partial agonist action of ZK 36374 The PGI_2 analogue, ZK 36374, produced a maximum contractile response lower than that obtained with PGE_2 (mean = 47%, range 33–75%, $n = 8$) (Figure 2a). The interaction of ZK 36374 with PGE_2, 16,16-dimethyl PGE_2, 11,9-epoxymethano PGH_2, $PGF_{2\alpha}$, PGI_2 and carbachol was also studied, by adding cumulative doses of one of these compounds to the organ bath in the presence of a fixed concentration of ZK 36374 (0.1 or 0.3 μg/ml). Typical results are shown in Figure 3. The contractile action of both 11,9-epoxymethano PGH_2 and carbachol was apparently additive with that of ZK 36374. However, ZK 36374 opposed the contractile action of PGE_2, 16,16-dimethyl PGE_2, $PGF_{2\alpha}$ and PGI_2. This activity of ZK 36374 is consistent with a partial agonist action at the PGE-sensitive system.

Estimates of the affinity constant of ZK 36374 were made using the log concentration-effect curves for PGE_2 and ZK 36374 obtained on the same preparation. It was assumed that PGE_2 had a high efficacy and that for submaximal responses receptor occupancy was both small and directly proportional to its concentration. The concentration of PGE_2 which gave a response equal to the ZK 36374 maximum response (100% occupancy by ZK 36374) was found first. The response which corresponded to half this concentration of PGE_2 was then found and finally the concentration of ZK 36374 which gave a response identical in size to it was read off. The reciprocal of the ZK 36374 concentration (corresponding to 50% occupancy) was the affinity constant. From six experiments the mean affinity constant was calculated to be $1.03 (\pm 0.17$ s.e.mean) $\times 10^8 M^{-1}$.

Effect of the thromboxane antagonist, EP 045 In a series of experiments the effect of EP 045 on the contractile action of several of the analogues was determined.

On control preparations three cumulative concentration-response relationships for PGE_2 or 11,9-epoxymethano PGH_2 were established at approximately hourly intervals. In the antagonist-treated preparations EP 045 at 1.3×10^{-6} M was added 5 min before the start of the second agonist sequence and further increased to 2.6×10^{-6} M 5 min before the start of the third sequence. EP 045 caused a pronounced parallel shift to the right of the 11,9-epoxymethano PGH_2 log concentration-response curve (Figure 4). Dose-ratios are shown in Table 2. From the Gaddum-Schild equation we obtain affinity constants of 6.9 (s.e.mean ± 0.9) $\times 10^6$ and 7.5 (± 0.7) $\times 10^6 M^{-1}$ respectively for the lower and higher concentrations of EP 045. EP 045 caused much smaller shifts of the PGE_2 concentration-response curves (Table 2). More determinations would be required to determine whether this small degree of antagonism is significant. The agonist action of ZK 36374 was not blocked by EP 045.

With 16,16-dimethyl PGE_2 the situation is more complex. The log concentration-response curve for 16,16-dimethyl PGE_2 over the concentration range of 0.1–20 ng/ml was parallel to that of PGE_2 (Figure 2), and remained constant during the experiment. Under these circumstances EP 045 (1.3×10^{-6} M) produced only a small shift (dose-ratios = 1.50 and 1.75) of the 16,16-dimethyl PGE_2 curve. At higher concentrations of 16,16-dimethyl PGE_2 (0.1–2.0 μg/ml) further small contractions could be elicited which eventually approached the 11,9-epoxymethano PGH_2 maximum (Figure 5a). However, in this situation some tachyphylaxis to the contractile action of 16,16-dimethyl PGE_2 occurred, such that subsequent concentration-response curves were shifted to the right, with slight lowering of the maximum response (Figure 5b). This made any effect of EP 045 on the high concentration component of

Table 2 Effect of EP 045 on contractile responses to different agonists on the bullock iris sphincter

Treatment	*Agonist*	*Number of tests*	*Dose-ratios for agonist cumulative sequences* (first sequence = 1.00) *Second*	*Third*
Control	PGE_2	4	0.86 ± 0.16	0.96 ± 0.18
	11,9-epoxymethano PGH_2	4	1.06 ± 0.07	1.27 ± 0.13
EP 045	PGE_2	3	1.16 ± 0.03	1.63 ± 0.13
	11,9-epoxymethano PGH_2	4	10.0 ± 1.2	20.5 ± 1.8
	ZK 36374	—	0.83, 1.10	1.00

With the EP 045-treated preparations the second agonist sequence was established in the presence of 1.3×10^{-6} M EP 045 and the third sequence in the presence of 2.6×10^{-6} M EP 045.

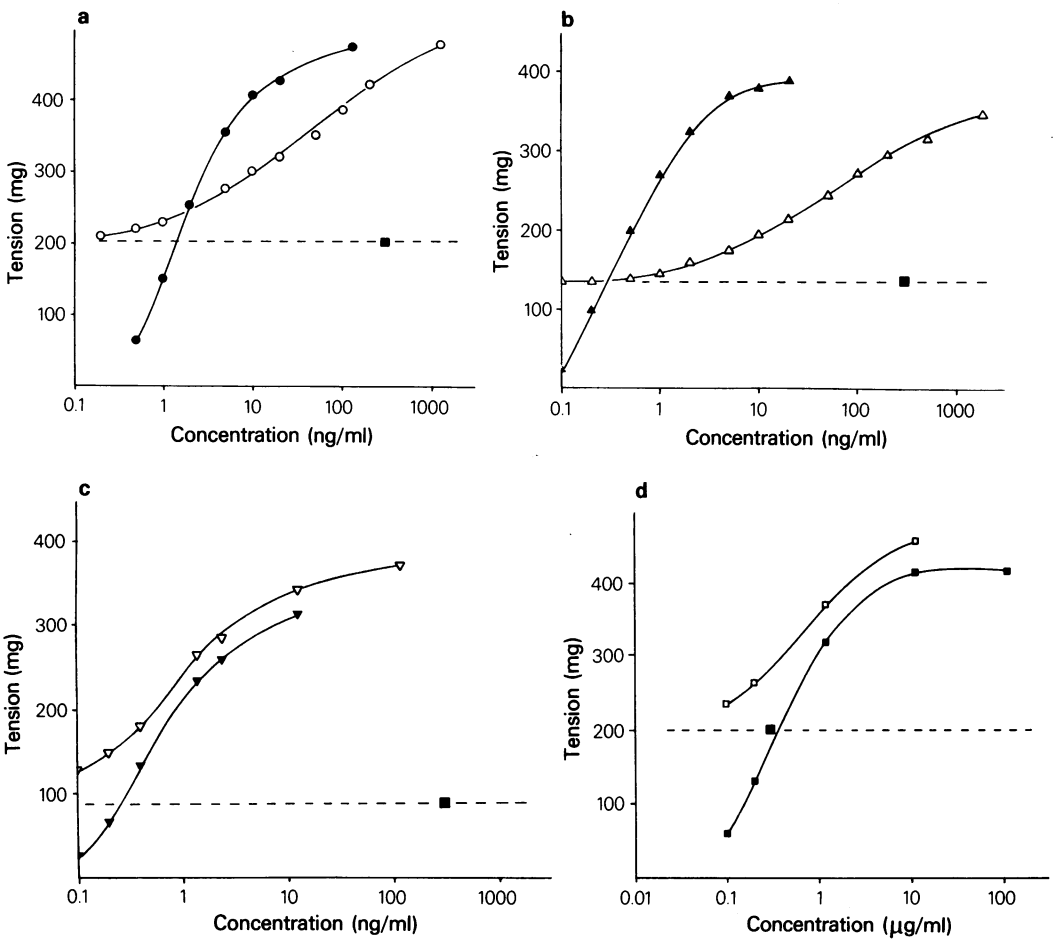

Figure 3 Bullock iris sphincter muscle: interaction of ZK 36374 with (a) PGE_2, (b) 16,16-dimethyl PGE_2, (c) 11,9-epoxymethano PGH_2 and (d) carbachol. In each instance a cumulative concentration-response relationship was first established to the full agonist alone (solid symbols), followed by a cumulative relationship (open symbols) in the presence of a fixed concentration of 0.3 μg/ml ZK 36374 (■).

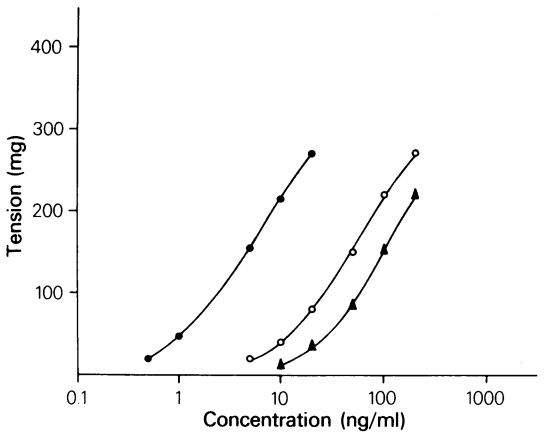

Figure 4 Bullock iris sphincter muscle: log concentration-response curves for 11,9-epoxymethano PGH_2 alone (●) and in the presence of 0.5 (○) and 1.0 (▲) μg/ml EP 045.

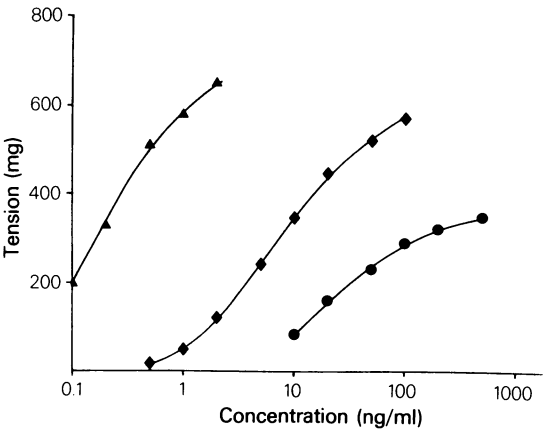

Figure 6 Bullock iris sphincter muscle log concentration-response curves for 11,9-epoxymethano PGH_2 (◆), EP 011 (▲), and 9, 11-ethano PGH_2 (●).

16,16-dimethyl PGE_2 action difficult to determine (Figure 5a).

Other thromboxane-like compounds Three compounds with thromboxane-like activity on other smooth muscle preparations were tested, 9,11-azo PGH_2, (±)-9,11-ethano PGH_2 and (±)-17, 18, 19, 20-tetranor-16-*p*-fluorophenoxy-9, 11-ethano PGH_2 (EP 011). Typical concentration-response curves are shown in Figure 6. EP 011 is the most potent contractile agent we have tested on the bullock iris sphincter and its action was both slow in onset and offset. The equipotent molar ratio (11,9-epoxymethano $PGH_2 = 1.0$) for EP 011 was 0.033 ± 0.008 ($n = 8$) and for 9,11-azo PGH_2 was 0.74 ± 0.15 ($n = 4$).

The 9,11-ethano PGH_2 analogue was less active than 11,9-epoxymethano PGH_2 and gave a maximum response between 50–80% of the EP 011 or 11,9-epoxymethano PGH_2 maxima (Figure 6). It opposed the contractile action of 11,9-epoxymethano PGH_2.

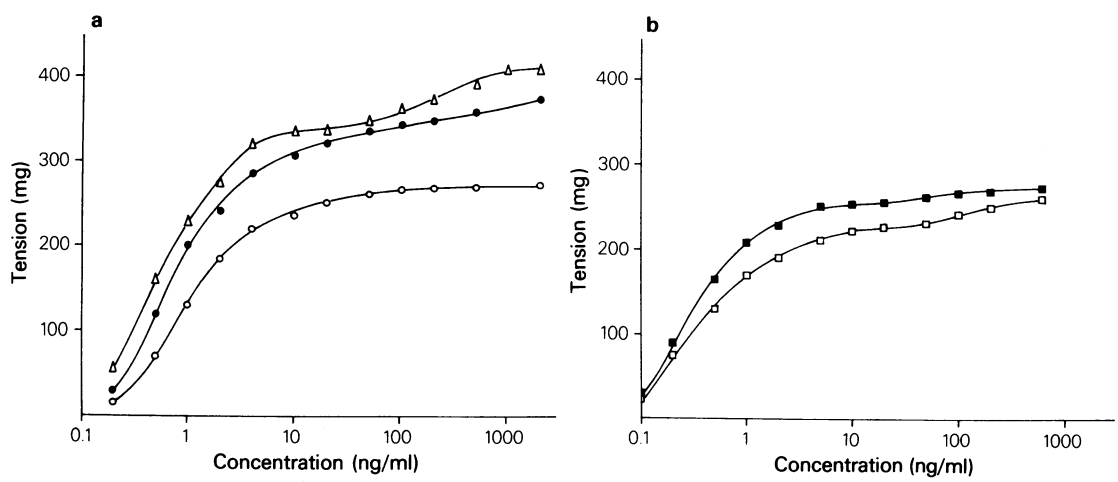

Figure 5 Bullock iris sphincter muscle: (a) Preparation A—log concentration-response curve for 16,16-dimethyl PGE_2 (△) showing distinctly the two components of the curve. Preparation B—concentration-response relationships for 16,16-dimethyl PGE_2 in the absence (●) and the presence (○) of EP 045 (0.5 μg/ml). (b) Preparation C—control tissue on which two consecutive cumulative concentration-response relationships for 16,16-dimethyl PGE_2 were established (■, □).

Dog iris sphincter muscle

A limited number of dog iris preparations were available for testing of the prostaglandin analogues. $PGF_{2\alpha}$ was a potent contractile agent producing a 50% maximum response at concentrations of 0.3–1.5 ng/ml. The relative activities of several other analogues are shown in Table 3. Of particular interest is the high activity of ICI 81008 and the low activity of 16,16-dimethyl PGE_2. ZK 36374 produced small contractions of the iris sphincter at concentrations of 100–500 ng/ml. The actions of ZK 36374 and $PGF_{2\alpha}$ were additive as shown in Figure 7.

On four preparations EP 045 at 500 ng/ml produced only small shifts of the $PGF_{2\alpha}$ log concentration-response curve (dose-ratios = 1.35, 1.45, 1.80 and 1.80).

Table 3 Activities of prostaglandin analogues on the dog isolated iris sphincter preparation

Compound	*Equipotent molar ratio* ($PGF_{2\alpha}$ = 1.0)
(±) 17,18,19,20-tetranor-16-*m*-trifluoromethylphenoxy $PGF_{2\alpha}$ (ICI 81008)	0.10, 0.19, 0.49
(±) 17, 18, 19, 20-tetranor-16-*p*-fluorophenoxy $PGF_{2\alpha}$ (ICI 79939)	0.24, 0.31, 0.86
PGE_2	60, 137, 230
16,16-dimethyl PGE_2	(> 1000), 530, 530
ZK 36374	(120), (> 100), (1200)
11, 9-epoxymethano PGH_2	(120), (230), (300)

Individual values are the result of a comparison with $PGF_{2\alpha}$ on a single preparation. Values in parentheses are derived from experiments in which a complete concentration-response curve for the test compound was not established; equipotent molar ratios relate to responses 20% of the $PGF_{2\alpha}$ maximum.

Discussion

Our results suggest that the bovine iris sphincter contains two types of prostanoid receptor. One is more sensitive to PGE analogues and the other to thromboxane/prostaglandin endoperoxide analogues.

The PGE-sensitive system responds to PGE_2 and its 16,16-dimethyl and 17,18,19,20-tetranor-16-*p*-chlorophenoxy analogues: PGE_1 is less active, and PGI_2 and PGD_2 show very weak activity. The system is further characterized by the unique action of ZK 36374. This analogue is a stable carbacyclin and shows properties similar to PGI_2 on bovine coronary and human platelets (Schrör, Darius, Matzky & Ohlendorf, 1981). Its potency is equivalent to PGI_2 in these latter tests. In the bovine iris sphincter preparation, ZK 36374 elicits threshold contractile effects at concentrations of 1–2 ng/ml whereas PGI_2 concentrations for similar responses are 10–30 times higher. Moreover, ZK 36374 appears to act as a partial agonist, giving a lower maximum than PGE_2 and opposing the actions of PGE_2, 16,16-dimethyl PGE_2, $PGF_{2\alpha}$ and PGI_2, but not those of 11,9-epoxymethano PGH_2 and carbachol.

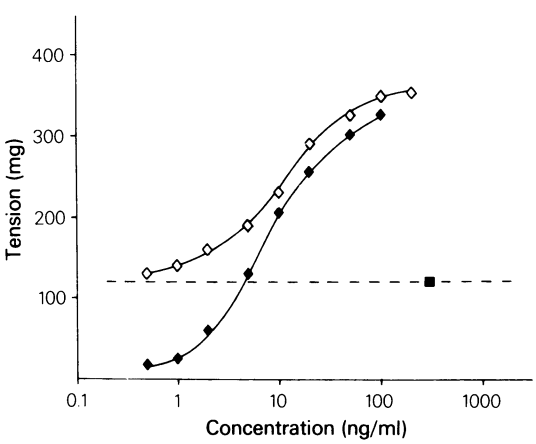

Figure 7 Dog iris sphincter preparation: log concentration-response curve for $PGF_{2\alpha}$ acting alone (◆) and the corresponding curve (◇) in the presence of 0.3 μg/ml ZK 36374 (■).

The other prostanoid-sensitive system shows properties similar to those found for the rabbit aorta and the dog saphenous vein (Jones, Wilson & Marr, 1979; Jones & Wilson, 1980). Thus the 11,9-epoxymethano PGH_2 analogue is a potent full agonist whose action is blocked by the semicarbazone analogue, EP 045 (Jones & Wilson, 1981). The affinity constant for EP 045 on the bovine iris sphincter ($7.2 \times 10^6\ M^{-1}$) is similar to those found previously for the 11,9-epoxymethano PGH_2/EP 045 combination on the dog saphenous vein ($2.2 \times 10^7\ M^{-1}$) and guinea-pig trachea ($3.3 \times 10^7\ M^{-1}$). EP 011 is a considerably more powerful agonist than 11,9-epoxymethano PGH_2 and shows a typically slow onset and a prolonged duration of action. The 9,11-ethano PGH_2 analogue is a partial agonist.

Weak thromboxane-like activity on smooth muscle has been previously demonstrated for 16,16-dimethyl PGE_2 (Jones & Wilson, 1980). Thus on the rabbit aorta and dog saphenous vein, 16,16-dimethyl PGE_2 is 33 and 55 times respectively less active than 11,9-epoxymethano PGH_2 in terms of contractile action. The additional contractions seen with high concentrations of the 16,16-dimethyl analogue on the bovine iris sphincter could be due to this type of thromboxane activity.

On the dog iris sphincter preparation a different

order of agonist potency was found. $PGF_{2\alpha}$ and its 16-*m*-trifluoromethylphenoxy analogue (ICI 81008) are potent agonists whereas PGE_2 and 11,9-epoxymethano PGH_2 show only weak activity. The very low activity of 16,16-dimethyl PGE_2 is in complete contrast to its high activity on the bovine iris sphincter. ZK 36374 is also a weak contractile agent and does not oppose the action of $PGF_{2\alpha}$. These results may indicate that a PGE-sensitive system similar to that found in the bullock iris sphincter is not present in the dog iris sphincter. It is possible that the activity of PGE_2 is due to its ability to interact with the $PGF_{2\alpha}$ canine receptor.

ICI 81008 is a potent luteolytic agent (Dukes, Russell & Walpole, 1974) mimicking the action of $PGF_{2\alpha}$. It shows similar activity to $PGF_{2\alpha}$ on the rabbit jejunum *in vitro* and the rabbit oviduct *in vivo* (Welburn & Jones, 1978). Its low activity on PGE-sensitive preparations has been documented previously – guinea-pig uterus *in vitro* (Dukes, Russell & Walpole, 1974) and guinea-pig ileum *in vitro* (Welburn & Jones, 1978).

Preliminary investigations have revealed, in addition to a thromboxane-sensitive system, a PGE-sensitive system in the guinea-pig isolated trachea with properties similar to those reported here for the bullock iris sphincter. It also appears that the receptors which mediate the excitatory actions of PGE_2 on smooth muscle are quite different in their structure-activity relationships from those which mediate the typical inhibitory effects of PGE_2, such as bronchodilatation and vasodilatation.

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