

ACTIVITY OF PROSTAGLANDIN E, F, A AND B  
ON SPHINCTER, DILATOR AND CILIARY  
MUSCLE PREPARATIONS OF THE CAT EYE

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Summary

Both sphincter and dilator muscle preparations of the cat iris contract to prostaglandins;  $F_{2\alpha}$  and  $E_2$  are the most potent and  $A_1$  and  $B_1$  the least. Ciliary muscle strips relax to PG's provided that the strips are precontracted.  $E_1$ ,  $E_2$  and often  $F_{2\alpha}$  are more potent relaxants than the remaining PG's. The effects of PG's are not altered by  $\alpha$  or  $\beta$  blockade nor by atropine; however, propranolol blocks the PG induced relaxation of the ciliary muscle. The effects of PG's on the sphincter are antagonized by catecholamines; but the latter act synergistically in contracting the dilator and in relaxing the ciliary muscle. Indomethacin markedly potentiates the effects of PG's on all three muscle preparations.

Abbreviations

PG: prostaglandin, Es: eserine, Ach: acetylcholine, Epi: epinephrine, norEpi: norepinephrine, DCI: dichloroisoproterenol.

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## Introduction

Prostaglandins may constrict the pupil as summarized in Table I:

Table I Effects of PG's on the Pupil

<u>Investigators</u>	<u>Effect</u>	<u>Species</u>	<u>PG</u>	<u>Route</u>
Waitzman and King <sup>1</sup>	miosis	rabbit	E <sub>1</sub> E <sub>2</sub> F <sub>1α</sub>	ic
	miosis	cat	E <sub>1</sub> E <sub>2</sub>	
Beitch and Eakins <sup>2</sup>	frequent miosis	rabbit	E <sub>1</sub> E <sub>2</sub>	ic
			F <sub>1α</sub> F <sub>2α</sub> A <sub>1</sub>	
Eakins <sup>3</sup>	miosis	cat	E <sub>1</sub> E <sub>2</sub>	ic
Starr <sup>4</sup>	none	rabbit	E <sub>1</sub> E <sub>2</sub>	ic and
				iv
Kelly and Starr <sup>5</sup>	none	monkey	E <sub>1</sub> E <sub>2</sub>	ic
Casey <sup>6</sup>	miosis	monkey	E <sub>2</sub> (+ topical atropine)	iv and topical

ic = intracameral (into the anterior chamber)  
iv = intravenous

The diameter of the pupil is a function of the tension in the sphincter and the dilator muscle. It is therefore not a priori possible to predict how PG's produce miosis unless their effects on sphincter and dilator are known. Since in vivo studies cannot easily separate these effects, studies on isolated muscle preparations are preferable. Also unknown is the effect of PG's on the ciliary muscle - if any. In this case isolated preparations are required<sup>7</sup> since in vivo studies are probably not feasible. This paper is limited to the effect of PG's on the isolated internal muscles of the eye of the cat.

## Methods

Eyes were enucleated from adult cats under pentobarbital anesthesia. Strips of sphincter, dilator and ciliary muscles were dissected as described previously.<sup>8</sup> Two strips were placed in a 30 ml muscle bath under adequate tension. (Table II)

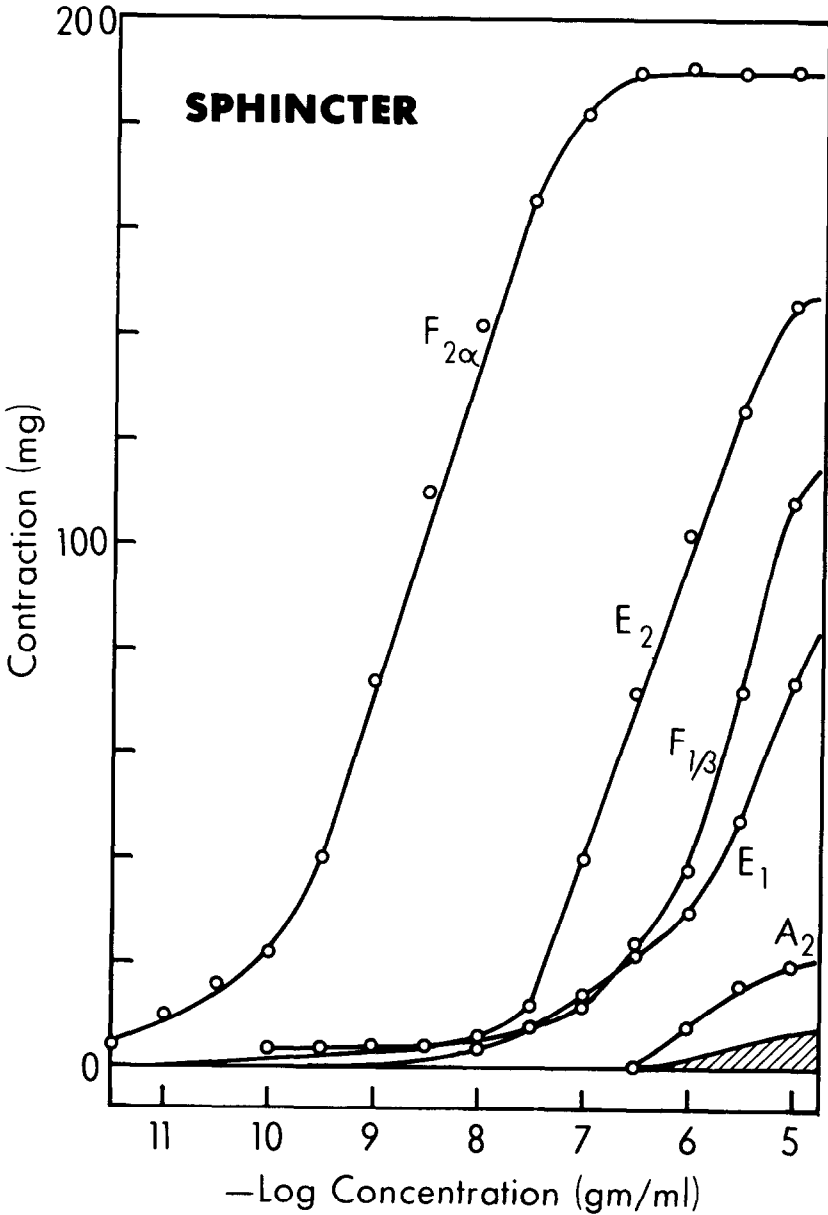


Fig. 1 Cumulative dose response curves. Shaded area refers to small contractions by  $PGA_1$ ,  $B_1$ , and  $B_2$ .

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Table II Number of muscle strips and tension applied in bath

	Number	Tension (mg)
Dilator muscle strips	73	50-75
Sphincter muscle strips	71	50-75
Ciliary muscle strips	79	250-300

The bath contained Krebs-Ringer Solution kept at 37° C and was supplied by 95% O<sub>2</sub>-5% CO<sub>2</sub>. Drug effects were measured under isometric conditions by means of Grass force displacement transducers (Model FT.03C). The responses were recorded on a Grass polygraph Model 7C.

Prostaglandins included E<sub>1</sub>, E<sub>2</sub>, F<sub>1β</sub>, F<sub>2α</sub>, A<sub>1</sub>, A<sub>2</sub>, B<sub>1</sub> and B<sub>2</sub>. They were dissolved in 2.5 ml of ethylalcohol, diluted with Ringer solution to a concentration of 1 mg per ml and stored frozen. Acetylcholine was used as the hydrochloride; L-epinephrine, L-norepinephrine and L-isoproterenol as bitartrate; atropine as sulfate; eserine as salicylate. Adrenergic blocking agents included dichloroisoproterenol, propranolol, tolazoline, phentolamine and phenoxybenzamine. To ensure blockade, the strips were exposed to 1 or 10 µg of these agents per ml bath fluid for at least 10 minutes. In some experiments, phenoxybenzamine 10 mg per kg was injected intravenously 1 hour before enucleation.

The weight of the salt of the drugs mentioned in the text pertains to the final concentration in the bath (weight/ml of bath solution).

### Results

1. SPHINCTER. The cat sphincter contracts to prostaglandin E, F, A, and B. A cumulative dose response curve for a single strip is shown in Fig 1. The sequence of potency was similar for 5 other sphincters in which the order of PG's added to the bath was varied. On one strip PGE<sub>1</sub> appeared more potent than PGE<sub>2</sub>.

If the sphincter is exposed to eserine, 1 µg/ml, it will slowly contract and subsequent Ach will produce a rapid superimposed contraction. If now the eserinated sphincter contracted by Ach, 0.1-0.01 µg/ml, is exposed to PG's another contraction ensues. This contraction may be stronger than before Es-Ach. Interestingly, some strips that did not respond to PG's contracted after the muscle had been eserinated and contracted still more after a previous contraction by Ach. An occasional sphincter relaxed with PG's- 3 out of 71 strips.

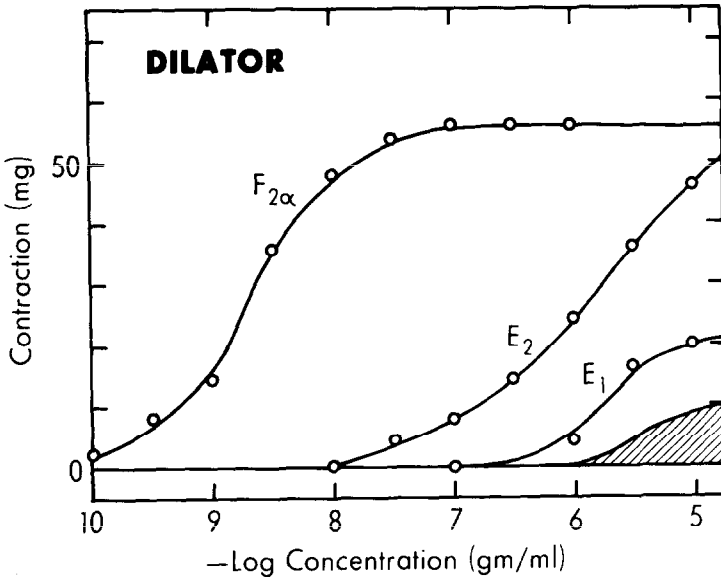


Fig. 2 Cumulative dose response curves. Shaded area refers to small contractions by  $PGA_1$ ,  $B_1$ ,  $A_2$ ,  $B_2$ , and  $F_{1\beta}$ .

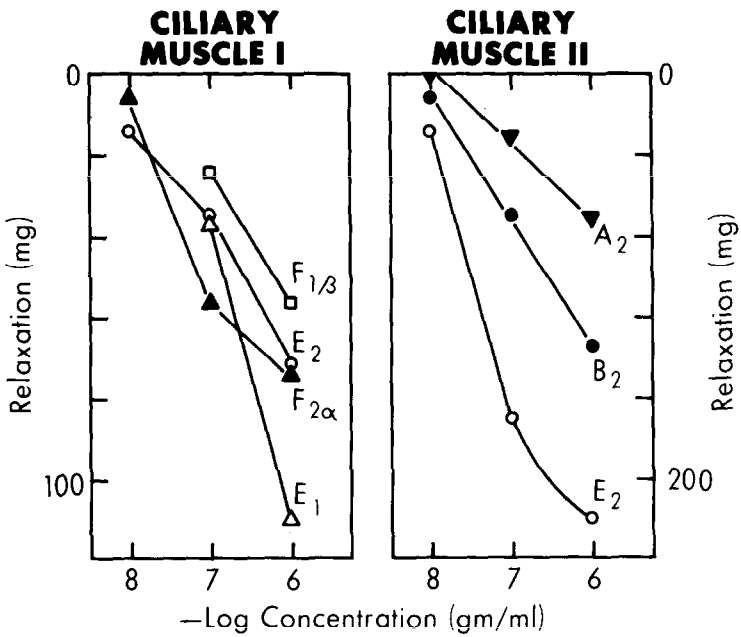


Fig. 3 Cumulative dose response curves.

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Catecholamines (epinephrine, 0.1  $\mu$ g; norepinephrine, 0.1  $\mu$ g; and isoproterenol, 0.01  $\mu$ g) relax the contraction produced by PG's. The relaxation is dose dependent with a  $\beta$ -sequence of potency. In order to establish whether this is due to functional antagonism or to inhibition of the PG effect, a series of experiments was done in which PG was given first, followed by epinephrine; after washing, epinephrine was given first followed by PG. The PG's used were E<sub>1</sub>, E<sub>2</sub>, and F<sub>2</sub> $\alpha$ . It appeared that the endlevel of contraction of the strip in 17 out of 21 experiments was higher with PG-Epi than with Epi-PG. The experiments were repeated after the muscle had been precontracted with Es-Ach. Again, the final level of contraction of the sphincter in 5 out of 6 strips was greater with PG-Epi than with Epi-PG.

In subsequent series of experiments with precontracted muscles, a low dose of PGE<sub>1</sub>, E<sub>2</sub>, or F<sub>2</sub> $\alpha$  was added to the bath which by itself just produced no contraction. Then the muscle was relaxed with epinephrine. There was no significant difference in the amount of relaxation by epinephrine with and without PG's.

The contraction produced by PG's is not blocked by atropine, 10 mg/ml. The same dose of atropine has no effect when given after a contraction produced by PG's whereas epinephrine, norepinephrine and isoproterenol produced strong relaxations.

The effect of PG's is not affected by  $\alpha$  or  $\beta$  blockade. The sphincter contracted to PG's after the animal had received 10 mg per kg of phenoxybenzamine intravenously before enucleation; and the contraction to PG's was not affected by either tolazoline or phentolamine after it had been added to the bath in a concentration of 1  $\mu$ g/ml. Propranolol or DC1, up to 10  $\mu$ g/ml, did not affect the contractions induced by PG's; subsequent relaxations by catecholamines were indeed reduced or blocked.

Indomethacin, 1  $\mu$ g/ml, frequently tripled the effect of PG's with or without a previous contraction with Es-Ach.

2. DILATOR. The dilator, unexpectedly, also strongly contracts to PG's; a dose response curve for a single muscle strip is shown in Fig 2. Two other strips revealed the same order of PG potency although on one strip the potency of E<sub>1</sub> was approximately equal to E<sub>2</sub>. Many strips received a single dose of different PG's and these single dose administrations repeatedly confirmed the order of potency of Fig 2.

If the dilator is first contracted by Epi or nor-Epi, PG's will produce a superimposed contraction. Conversely, if PG's are added first, subsequent epinephrine will produce another contraction. Like the sphincter, Epi-PG produced consistently a greater contraction than PG-Epi in 12 out of 14 strips thus indicating again that the interaction is non-additive. The PG's investigated were limited to E<sub>1</sub>, E<sub>2</sub> and F<sub>2</sub> $\alpha$ . As was the case for the sphincter, a subthreshold dose of PG's (which by itself produced no contraction) did not affect the final level of contraction produced by Epi or nor-Epi.

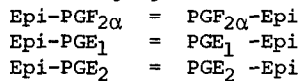
Atropine, up to 10 µg/ml, does not block the effect of PG's and neither does it block the effect of catecholamines. DCI and propranolol, 1 µg/ml, strongly potentiate the effect of catecholamines as described in a previous paper<sup>8</sup>; but these agents neither block nor enhance the effect of PG's.

Phenoxybenzamine before enucleation completely blocks the effects of Epi and nor-Epi but the dilator still contracts to PG's. Tolazoline and phentolamine added to the bath either block or depress the effect of Epi and nor-Epi but again do not alter the effect of PG's.

Indomethacin added to the bath hardly affects the tension of the muscle; but it strongly potentiates the effect of PG's by either doubling or tripling its contractions.

3. CILIARY MUSCLE. In initial experiments, ciliary muscle preparations appeared completely refractory to all PG's investigated. In later experiments, the muscle was precontracted by Es-Ach; under these conditions a subsequent dose of PG's relaxed the strips. Cumulative dose response curves are shown in Fig 3, measured on two different strips. The number of determinations that can be done on a single ciliary muscle strip is limited and less than on sphincter and dilator strips. After several doses of PG's, the relaxation response sometimes rapidly diminishes, possibly due to tachyphylaxis. By comparing 12 precontracted strips, each of which was relaxed by at least 3 doses of 2 PG's, the order of potency is tentatively ( $F_{2\alpha}$ ) -  $E_1$  -  $E_2$  - ( $F_{2\alpha}$ ) -  $F_{1\beta}$  -  $B_2$  -  $A_2$ . Notable exceptions: on 2 strips the sequence was  $E_2$  -  $B_2$  -  $A_2$  -  $F_{2\alpha}$  and on 1 strip  $E_2$  was more potent than  $E_1$ . In three single dose comparisons between  $E_1$ ,  $E_2$ , and  $F_{2\alpha}$ ,  $F_{2\alpha}$  was the least effective.

Catecholamines relax the precontracted muscle with a  $\beta$  sequence of potency; more relaxation may be seen from catecholamines after initial relaxations due to PG's. However, the final level of relaxation was equal with the following agents:



Blockade by phentolamine, 1 µg/ml, did not affect the relaxation of the contracted muscle by PG's but it did suppress the relaxation by catecholamines. However, propranolol, 10 µg/ml, effectively antagonized the relaxation by PG's and, expectedly, blocked also the relaxation by catecholamines.

Indomethacin contracts the ciliary muscle. It strongly potentiated the relaxation by PG's by a factor 2 to 3.

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## Discussion

The antagonism of the dilator and sphincter, both contracting to PG's was unexpected for antagonism of the iris muscles was not present with respect to catecholamines. In earlier work<sup>9</sup> we demonstrated dual receptors in each muscle with a predominance of  $\beta$  receptors in the sphincter and  $\alpha$  receptors in the dilator. Hence in the cat, activation of the adrenergic receptors of the iris muscles would produce essentially synergistic effects and it was therefore surprising to find antagonism with respect to PG's. On the other hand, in the monkey, the two muscles respond antagonistic to adrenergic agents; this demonstrates that antagonism also occurs with other agents and is not a peculiarity of PG's.

The question arises whether PG's can produce miosis at all in view of the antagonism just mentioned. One can estimate the PG induced in vivo tension in the iris muscles from the in vitro tension of the excised strips as follows. The excised sphincter strip measures about one third of the whole sphincter; the excised dilator (3mm in width) measures about one tenth of the whole dilator strip. Hence the tension in the entire sphincter approximately equals the tension in the sphincter strip multiplied by a factor 3, while the tension in the whole dilator is found from the tension in the dilator strip multiplied by a factor 10. If one calculates in this way the tension in the iris muscles induced by  $\text{PGF}_{2\alpha}$ ,  $E_1$  and  $E_2$  at each integer PG level of Fig 1 and 2 the results are close but the sphincter muscle tension appears higher in 9 out of 11 samples; thus based on these estimates miosis of the pupil in vivo would indeed be the most likely result with these agents.

In vivo experiments in the rabbit and cat revealed that the miotic effect of  $\text{PGE}_1$  on the pupil was counteracted by isoproterenol. This was considered to be physiologic antagonism although not backed up by rigorous proof.<sup>10</sup> It is now clear that in the cat, antagonism may operate with respect to the sphincter but the dilator response is synergistic. Strictly speaking, not even the sphincter is purely antagonized; for both Epi and nor-Epi frequently produce a slight initial contraction ( $\alpha$  activation) before a pronounced relaxation occurs. This is confirmed by  $\beta$  blockade.

The antagonism of catecholamines on the sphincter is presumably pharmacologic and not entirely physiologic; neither is the synergism on the dilator. For we have repeatedly found that the endlevel of contraction of the muscle depends on the sequence of drug administration, with a lower level attained after Epi-PG than after PG-Epi. However, if there were drug interaction one would expect an effect of a sub-threshold dose of PG's on catecholamines, which we were unable to demonstrate. Possibly the 2 minute exposure to PG's was too short.

PG's relaxed the ciliary muscle but only after the muscle had been precontracted; this was done by either Es-Ach or by pilocarpine. To exclude the remote possibility of cholinergic inhibition by PG's, an attempt was made to precontract the muscle with KCl. However, after



KCl, there was no response or at the most a slight contraction. Serotonin contracts the sphincter; but we earlier noted that serotonin has no effect on the ciliary muscle.<sup>β</sup> Hence a direct effect of PG's on the ciliary muscle could not be demonstrated except for occasional small relaxations by PGE<sub>1</sub> on the non-contracted muscle. Otherwise, in the precontracted ciliary muscle the relaxation by PG's parallels the relaxation by catecholamines. In this case, the effect is additive; the endlevel of contraction is the same irrespective of the sequence of administration of PG's and catecholamines.

Neither atropine nor  $\alpha$  or  $\beta$  blockade had any effect on PG activity although a high dose of propranolol, 10  $\mu$ g/ml, antagonized the PG induced relaxation of the ciliary muscle. Since this dose is high, we doubt that it proves PG activity on  $\beta$  receptors of the ciliary muscle.

Indomethacin clearly potentiates the effect of PG's on sphincter, dilator and ciliary muscle, the potentiation being in the order of a factor 2 to 3. Indomethacin may also have a direct effect on the muscle. It strongly relaxes the sphincter, has no effect on the dilator and contracts the ciliary muscle. However, the changes in tension do not account for the potentiation. The relaxation of the sphincter by indomethacin can be counteracted by Es-Ach and the contraction of the ciliary muscle by an initial dose of catecholamines. Under these conditions, the PG effects on both muscles are still potentiated by about the same amount. Further, the tension on the muscle can be mechanically adjusted and the potentiation remains essentially the same. Hence the effect is real and the potentiation is not due to a shift on the muscle tension curve. Moreover, PG effects on the dilator are also strongly potentiated by indomethacin, although this agent by itself produces no change in muscle tension. Since indomethacin inhibits the biosynthesis of PG's, PG precursors may interfere with PG receptors or directly depress the response of intraocular smooth muscle. In other words, the effect of PG's in the presence of indomethacin possibly reflects a more "pure" effect of PG's.

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