*Exp. Eye Res.* (1988) **47**, 761–769

# Morphological Study of the Anterior Segment of Cynomolgus Monkey Eyes Following Treatment with Prostaglandin $F_{2\alpha}$

ELKE LÜTJEN-DRECOLL* AND ERNST TAMM

*Department of Anatomy, University of Erlangen-Nürnberg, Krankenhausstr. 9, D-8520 Erlangen, West Germany*

(*Received 25 March 1988 and accepted in revised form 13 April 1988*)

Topically applied prostaglandin $F_{2\alpha}$ has been shown to lower intraocular pressure in cynomolgus monkeys. In this study the morphological changes, following topical treatment with 4–50 $\mu$g of prostaglandin $F_{2\alpha}$ for 4–8 days, were investigated. Semiquantitation of (1) accumulation of white blood cells as one sign of inflammation, (2) edema and (3) enlarged spaces between ciliary muscle cells were performed. Eighty sections per eye encompassing the whole circumference were investigated. No accumulation of white blood cells was seen in any of the eyes. Slight edema in the most anterior part of the ciliary processes occurred in most eyes, but only in part of the circumference. These changes could be either directly induced by the prostaglandin treatment or secondary to the decrease in intraocular pressure. The most pronounced change was the dilatation of the intramuscular spaces. These enlarged spaces could explain the physiologically shown increase in uveoscleral outflow.

*Key words:* light microscopy; prostaglandin $F_{2\alpha}$; ciliary process; ciliary muscle; uveoscleral flow; cynomolgus monkey.

## 1. Introduction

It has been shown that topical application of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) significantly reduces intraocular pressure (IOP) in rabbits (Camras, Bito and Eakins, 1977; Lee, Podos and Severin, 1984), cats (Stern and Bito, 1982; Bito, Draga, Blanco and Camras, 1983; Lee, Podos and Severin, 1984), monkeys (Camras and Bito, 1981; Stern and Bito, 1982; Bito, Draga, Blanco and Camras, 1983; Lee, Podos and Severin, 1984) and man (Guiffre, 1985). Recent physiological studies of cynomolgus monkeys have indicated that in this species the decrease in IOP is the result of increased uveoscleral outflow (Nilsson, Stjernschantz and Bill, 1987). This finding is supported by the fact that the decrease in IOP can be prevented by preceding the application of $PGF_{2\alpha}$ with pilocarpine, which is known to inhibit uveoscleral flow (Crawford and Kaufman, 1987). We now report on the morphological changes in the same species treated for 4–8 days with $PGF_{2\alpha}$ in a treatment protocol similar to that used in the physiological studies.

## 2. Materials and Methods

Seven young adult cynomolgus monkeys were treated by the topical application of $PGF_{2\alpha}$ to one eye for 4–8 days and the application of diluent to the other eye (Table I). The right eyes of two monkeys (R144/85, R145/85) were treated twice daily with 50 $\mu$g of the tromethamine salt, and the right eye of one other monkey (R15/87) was treated twice a day with 5 $\mu$g of the isopropylester. In these animals all doses were given as 2-$\mu$l drops applied to the central cornea of the supine monkey with blinking prevented between drops and for

* To whom correspondence should be addressed.

0014–4835/88/110761+09 $03.00/0

TABLE I

*Protocol of the prostaglandin $F_{2\alpha}$ treatment*

| No. | Duration of treatment (days) | Dose/ treatment ($\mu$g) | Time difference between last treatment and measurement (hr) | No. of doses | IOP before enucleation | Fixation |
|---|---|---|---|---|---|---|
| R145/85* | 4 | 50‡ | 3 | 6 | od 6 mmHg<br>os 13 mmHg | Perfusion |
| R144/85* | 4 | 50‡ | 5 | 6 | od 6 mmHg<br>os 13 mmHg | Immersion after dissection |
| R31/87† | 4 | 4§ | 6 | 7 | od 7·5<br>os 5 | Perfusion |
| R15/87* | 5 | 5§ | 4, 5 | 9 | od 4 mmHg<br>os 15 mmHg | Immersion, fixed intact |
| R32/87† | 7 | 4§ | 4 | 9 | od 12·5<br>os 5 | Immersion, fixed intact |
| R33/87† | 7 | 4§ | 4 | 9 | od 7·5<br>os 5 | Immersion fixed intact |
| R34/87† | 8 | 4§ | 4 | 11 | od 7·5<br>os 5·5 | Perfusion |

* Treated by Prof. P. L. Kaufman, Madison, Wis; IOP measured with Goldman tonometer.
† IOP measured with Schiøtz tonometer (Schiøtz readings with 7·5 g).
§ $PGF_{2\alpha}$-Isopropylester.
‡ $PGF_{2\alpha}$-tromethamine salt.

30 sec after the last drop. All of these treatments were administered by Dr Kaufman at the University of Madison, Wi, following the protocol described elsewhere (Crawford, Kaufman and True Gabelt, 1987). The IOP in these eyes was then measured by Goldmann applanation tonometry (Table I).

The right eyes of the remaining four monkeys (R31/87, R32/87, R33/87, R34/87) were treated with 4 $\mu$g of $PGF_{2\alpha}$ isopropylester (115 $\mu$g ml$^{-1}$) once daily. The animals, which were anesthetized with ketamine (10 mg kg$^{-1}$ i.m.), received in a clinical manner one 35-$\mu$l drop, covering the whole cornea and conjunctive of the eyes. With these monkeys under ketamine anesthesia (10 mg kg$^{-1}$ i.m.), their IOP was measured with a Schiøtz tonometer (7·5 g). After two doses, there was an IOP decrease of 2·5–4 units at 4 h after the last treatment of these monkeys. The treatment was then continued with the same dose, but twice daily. Before enucleation, the pressure difference between the treated eyes and the untreated, contralateral control eyes was 2–2·5 units in three of the animals (R31/87, R33/87, R34/87). According to the Friedenwald Calibration Scale, the pressure differences were similar to those in the eyes measured with the Goldmann tonometer. Only one monkey (R32/87) showed a difference of 7 units (17 mmHg) 4 h after the last treatment.

In all monkeys, slit-lamp examination of the eyes was performed before the IOP measurement, by a trained ophthalmologist. Neither flare nor cells were observed in the aqueous in any of the animals.

After the final measurement, three of the monkeys (R145/85, R31/87, R34/87) were fixed by perfusion via the heart with Ito's fixative (Ito and Karnovsky, 1968), following perfusion with heparinized NaCl solution (Table I). In three of the animals (R15/87, R32/87, R33/87) the eyes were fixed by immersion, immediately after enucleation (Table I). Windows were cut in the posterior sclera and the cornea of these eyes, after which the entire eyes were placed in Ito's fixative for a few hours to preserve the architecture of the ciliary muscle and its posterior insertion into Bruch's membrane (Lütjen-Drecoll, Tamm and Kaufman, in press).

The eyes of one of the animals (R144/85) treated with the tromethamine salt were bisected equatorially immediately after enucleation, immersion-fixed in Ito's fixative and then sent to us in West Germany in the same fixative (Table I). Anesthesia for in vivo enucleation and systemic perfusion was i.m. ketamine HCl 15 mg $kg^{-1}$, followed by i.m. pentobarbital Na 30 mg $kg^{-1}$. The animals were killed by pentobarbital overdose. These experiments conformed to the ARVO Resolution on the Use of Animals in Research (Association for Research in Vision and Ophthalmology, 1983).

The eyes were then prepared for light and electron microscopy. To ensure that the ciliary muscle remained anchored anteriorly and via Bruch's membrane posteriorly, two sectors running from the anterior to the posterior pole and having a width of approx. 5 mm at the equator were cut from opposite sides of the eyes that had been perfusion-fixed and from those that had been immersion-fixed with windows. These specimens were embedded in paraffin and 6-$\mu$m sections were cut and stained with Crossmon's stain (Crossmon, 1937). The rest of the globe was divided at the ora serrata and small pieces containing the whole thickness of the ciliary body, iris, adjacent cornea and sclera were embedded in Epon. After polymerization, 1-$\mu$m sections were cut with the ultramicrotome. These semithin sections were stained for reticular fibers with Movat's stain (Movat, 1961). Some of the paraffin sections were stained with Alcian Blue and a combined Alcian Blue–PAS staining for proteoglycans.

In 20 sections from each quadrant of all treated and untreated eyes (80 sections per eye), the following changes in the different tissues were looked for in every section:

(1) Accumulation of leucocytes and lymphocytes as a sign of inflammation (inflammation in Table II).

(2) Separation of the ciliary epithelium from the underlying capillaries or separation of the neural retina from the pigment epithelium (slight edema in Table II).

(3) Greeff's vesicles (Greeff, 1894).

(4) Enlargement of spaces between the ciliary muscle bundles in different parts of the ciliary muscle.

Table II gives the percentage of sections showing the above-mentioned criteria in the treated eyes. The quantification was done blind by E.L.D. and E.T. independently. There was no appreciative difference between the results of both investigators.

## 3. Results

Eighty sections from all parts of the circumference of each of the seven treated eyes were studied. No accumulation of white blood cells was seen. There was a slight separation of the ciliary epithelium in the anteriormost part of the ciliary processes separating both layers of the epithelium from the underlying capillaries (Fig. 1). This separation was not seen in all parts of the circumference, but rather only in 20–63% of the sections (Table II). In the one eye that was treated for 8 days, no such separation was observed. Greeff's vesicles were only seen in two eyes (treated for 4- and 5 days), one immersion- and one perfusion-fixed (Fig. 2B). In these two eyes, 33- and 25% of the sections respectively showed the vesicles. No edema was observed in the retina of any animal.

A distinct finding was the enlargement of the spaces between the ciliary muscle bundles (Figs 2B, 3). The single muscle cells were very thin (Fig. 3) and the muscle as a whole showed a relaxed appearance with no circular portion visible. In the four animals treated for 5–8 days, this feature was present in all sections in the longitudinal portion and in parts of the reticular portion. In the reticular part, the spaces were mainly around the nerve bundles and the larger vessels. In the longitudinal part, spaces were seen between the single fibre bundles. Following Alcian Blue–PAS staining for proteoglycans, no staining was observed in these spaces. With Movat's staining for reticular fibres in the control eyes, intense staining was seen

TABLE II

*Semiquantitative evaluation of the histological changes following $PGF_{2\alpha}$ treatment*

| No. | Duration of treatment (days) | Enlarged spaces between muscle fibres at the tip % | Enlarged spaces between muscle fibres in the longitudinal and reticular portion | Muscle contracted | Inflammation | Greeff's vesicles (%) | Slight edema | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Ciliary processes, anterior most portion (%) | Retina |
| R145/85 | 4 | 85 | - | + | | 33 | 33 | - |
| R144/85 | 4 | 30 | | ++ | | — | 50 | |
| R31/87 | 4 | 16 | 84% | (+) | — | - | 63 | |
| R15/87 | 5 | | 100% (wide) | — | | 25 | 25 | - |
| R32/87 | 7 | - | 100% (mainly around the vessels) | — | | — | 20 | - |
| R33/87 | 7 | - | 100% (mainly around the vessels) | - | - | - | 50 | |
| R34/87 | 8 | - | 100% (mainly around the vessels) | - | | — | | — |

Eighty sections from each eye were evaluated. The numbers represent the percentage of positive findings.
+, Slightly contracted muscle with a small circular portion. ++, Marked contraction with a well-developed circular portion.

FIG. 1. Sagittal section through the anterior part of the pars plicata of the ciliary body (Crossmon's stain. ×315), following treatment with $PGF_{2\alpha}$ for 5 days. The pigmented epithelium is partly separated from the stromal layer, indicating a slight edema (arrows).

surrounding the ciliary muscle bundles in all parts of the ciliary muscle. In the treated eyes, there was a loss of reticular fibre staining in the longitudinal and outer reticular part of the ciliary muscle. The inner reticular portion, as well as the circular portion, still stained. In two of the animals treated for 4 days (R145/85, R31/87), enlarged spaces were seen mainly in the anterior part of the ciliary muscle. Only in one monkey (R144/85), was the enlargement of the spaces less pronounced, even in the longitudinal portion. Interestingly, in this monkey the eyes was cut equatorially after enucleation and then immersion-fixed. As we have shown in a previous paper, this kind of preparation does not preserve normal muscle architecture; the muscle moves anteriorly and acquires a contracted appearance (Lütjen-Drecoll, Tamm and Kaufman, in press).

In the control (left) eyes, no reduction of IOP after treatment of the contralateral eyes with $PGF_{2\alpha}$ was observed. The morphology of these eyes showed no significant differences compared with normal eyes of the same age group (Fig. 2A).

## 4. Discussion

Morphological studies of the anterior segment of eyes following topical treatment with prostaglandin $F_{2\alpha}$ have not been undertaken until now. Previous studies in rabbits using prostaglandin $E_1$ and $E_2$ showed a marked plasma leakage following topical application of a very high dosage [5 mg in 10 drops (Pedersen, 1975a, b); 200 $\mu$g in two drops (Vegge, Neufeld and Sears, 1975)]. In a dosage of about 50 $\mu$g, Tamura (1974) found only a slight opening of the tight junctions. Similar findings

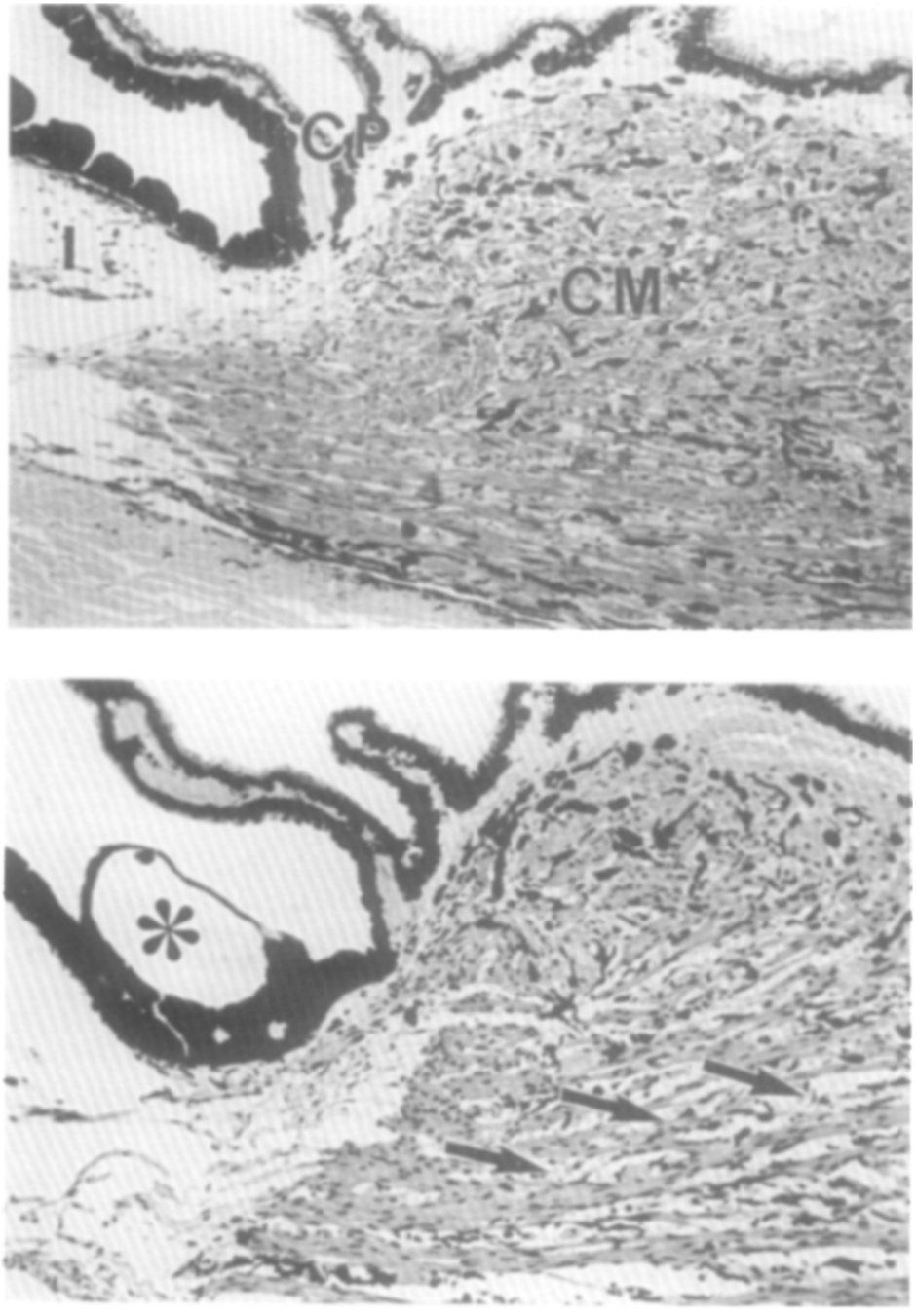


FIG. 2. Sagittal section through the anterior portion of the ciliary body (Crossmon's stain, × 135). (A) Vehicle-treated control eye. CM, Ciliary muscle; I, iris, CP, ciliary processes. (B) Following treatment with $PGF_{2\alpha}$ for 4 days, enucleation 4.5 h after the last treatment. Note the enlarged spaces between the thin muscle fibre bundles in the prostaglandin-treated eye (arrows). No edema of the ciliary processes is seen in this section. Asterisk: Greeff's vesicle.

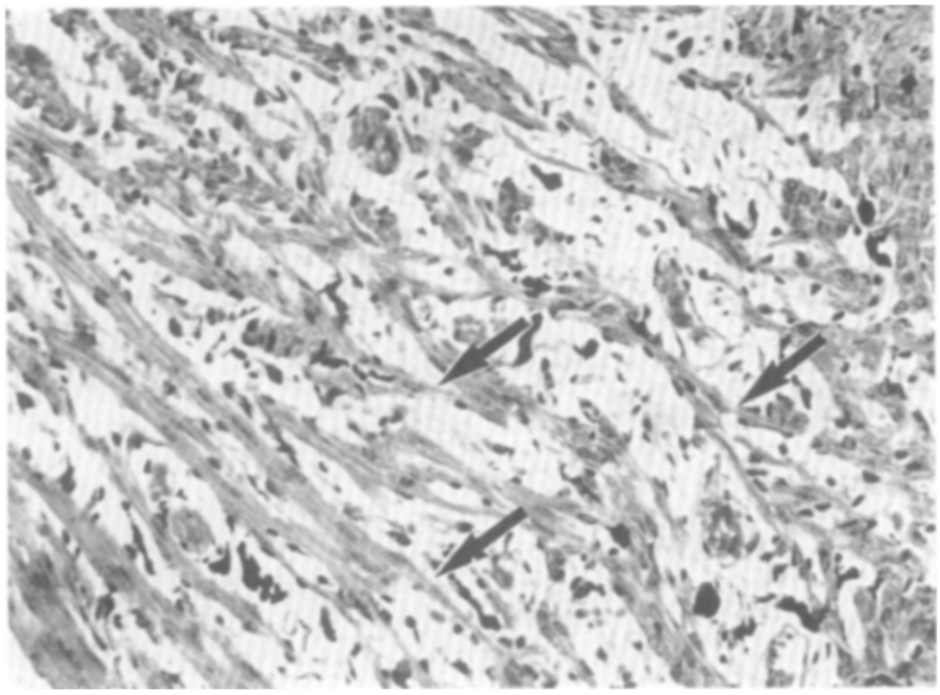

FIG. 3. Higher magnification of the ciliary muscle in Fig. 2B, showing the very thin muscle cells (arrows) and the wide intramuscular spaces. ×240.

with low doses of arachidonic acid were shown by Noske and Hirsch (1986) and Noske, Montcourrier, Keller, Arguillere and Hirsch (1986). All these changes were only observed, however, in the iridial processes of the rabbit eye, while the main processes in this species showed almost no change. Iridial processes do not exist in the primate ciliary body and until now it is not clear which part of the ciliary body could correspond morphologically or functionally to the rabbit ciliary processes. The only morphological study done in monkey eyes following $PGE_1$ showed an edema in the most anterior part of the ciliary processes (Okisaka, 1976).

In our study we also observed a slight separation of the ciliary epithelium from the underlying capillaries in the most anterior part of the ciliary processes. In contrast to Okisaka's findings (1976), we did not see this change within the whole circumference of the ciliary body, but only in 20–63 % of the sections. Histologically, the main part of the processes did not show signs of edema. We also did not see edema within the retina. Until now, we have not investigated whether the junctions in the non-pigmented epithelium are leaky. No aqueous flare was observed, indicating at least that no severe leakage had occurred.

From our findings we cannot exclude that prostaglandin $F_{2\alpha}$ in a dosage of 4–50 $\mu$g leads to a vascular leakage in the anteriormost parts of the ciliary processes. On the other hand, we described similar changes following treatment with cytochalasin B (Svedbergh, Lütjen-Drecoll, Ober and Kaufman, 1978). There we discussed the occurrence of edema in the anterior part of the processes as caused by a kind of internal paracentesis due to a sudden reduction in aqueous outflow resistance. The significant increase in uveoscleral flow and subsequent lowering of IOP is the main

physiological finding following application of $PGF_{2\alpha}$. It could be that the slight edema in the anterior ciliary processes is secondarily caused by this increase in aqueous outflow.

The most striking morphological changes observed in the treated eyes compared with the control eyes were the empty spaces between the muscle fibre bundles in the longitudinal portion and the loss of reticular fibres and ground substance in these enlarged spaces. This increase in the intramuscular spaces could be responsible for the observed increase in uveoscleral outflow. The reason for the loss of extracellular material is not known. Loss of reticular fibres surrounding the muscle bundles could result from collagenolytic activity in the aqueous humour. A similar increase in lytic activity as a consequence of increased prostaglandin activity has been described in other organs, e.g. in the cervix uteri (Uldbjerg et al., 1981; Ekman, Uldbjerg, Malmström and Ulmsten, 1983). Our present histological investigations do not clarify the origin of such possible lytic enzymes. We did not observe any accumulation of white blood cells as a sign of inflammation. However, single leucocytes and macrophages, as well as mast cells, can always be found between the muscle bundles. In addition, it is known that fibroblasts are also able to produce collagenases (for review, see Harris, Welgus and Krane, 1984).

If the loss of extracellular material within the ciliary muscle is responsible for the increase in uveoscleral flow, the question arises why that increase is transient in monkeys. One possibility is that the extracellular material can be rebuilt in a short time. Another possible explanation is that the thinning or relaxation of the muscle fibres may directly or indirectly be caused by prostaglandins, and that this relaxation is reversed some hours after the last treatment, so that the muscle thickens again. This and recovery of muscle tone might close the empty spaces and thereby close the uveoscleral pathways. Indeed, it has been reported that $PGF_{2\alpha}$ effectively reduces tension in preconstricted ciliary muscle preparations in vitro (van Alphen, Wilhelm and Elsenfeld, 1977). We have undertaken a new set of experiments to clarify these questions.

## ACKNOWLEDGMENTS

We would like to thank Dr J. Stjernschantz of Pharmacia A B, Uppsala, Sweden, for providing the prostaglandins used in this study. We would also like to thank Dr S. Jikihara, guest from the Department of Ophthalmology, Gifu University, Gifu, Japan, for the slit-lamp investigation of some of the monkeys and Eva Jakob, Karin Junge and Marco Göβwein for their expert technical assistance. This study was supported by Grant No. Dr 2-4 from the Deutsche Forschungsgemeinschaft.

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