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Increased Uveoscleral Outflow as a Possible Mechanism of Ocular Hypotension Caused by Prostaglandin $F_{2\alpha}$-1-Isopropylester in the Cynomolgus Monkey

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The effects of topical application of a single dose of prostaglandin $F_{2\alpha}$, administered as the isopropylester, on the intraocular pressure (IOP), aqueous humor flow (AHF), conventional, and uveoscleral outflow were studied in cynomolgus monkeys under pentobarbital anesthesia. 1 μg $PGF_{2\alpha}$ decreased the IOP by $2{\cdot}9 \pm 0{\cdot}6$ mmHg (3 hr after the application) as compared with the vehicle-treated control eye. The mean AHF during the whole experiment was slightly higher in the experimental than in the control eye, $1{\cdot}34 \pm 0{\cdot}11$ μl min^{-1} compared with $1{\cdot}16 \pm 0{\cdot}09$ μl min^{-1}. The uveoscleral outflow was significantly increased in the $PGF_{2\alpha}$-treated eye, $0{\cdot}98 \pm 0{\cdot}12$ μl min^{-1} compared with $0{\cdot}61 \pm 0{\cdot}10$ μl min^{-1} for the control eye. The conventional outflow was lower in the experimental eye throughout the experiment. Topical application of 10 μg pilocarpine at the time when the fall in IOP was expected prevented the drop in the IOP. Simultaneously the increase in the uveoscleral outflow was abolished. After systemic pretreatment with atropine, 1 mg (kg body weight)$^{-1}$ i.v., there was no significant difference in IOP, AHF, conventional or uveoscleral outflow between the $PGF_{2\alpha}$-treated, and the control eye. The results of the present investigation suggest that $PGF_{2\alpha}$ decreases the intraocular pressure by increasing the uveoscleral outflow. The mechanism behind the increase in the uveoscleral outflow remains to be established. Relaxation of the ciliary muscle as well as enlarged intramuscular spaces and loss of extracellular material may contribute to the effect.

Key words: aqueous humor flow; atropine; ciliary muscle; conventional outflow; cynomolgus monkey; intraocular pressure; pilocarpine; prostaglandin $F_{2\alpha}$; uveoscleral outflow.

1. Introduction

In rabbits, prostaglandins (PGs) can cause either an increase or a decrease in the intraocular pressure (IOP). Starr (1971) observed that, after intracameral administration, the well-known hypertensive phase sometimes was followed by a hypotensive phase of longer duration. Later studies have shown that the normal pattern of response, to topical application of PGs in rabbits, is indeed an initial increase in IOP followed by a more long-lasting decrease (Camras, Bito and Eakins, 1977; Lee, Podos and Severin, 1984). The initial hypertensive phase, which is more marked at higher doses, is associated with miosis, breakdown of the blood aqueous barrier (BAB), and protein leakage into the aqueous humor. As high doses and rabbits were commonly used in the early studies on the ocular effects of PGs, the attention was focused on the involvement of PGs in ocular inflammation (see Eakins, 1977).

In other species PGs cause ocular hypotension without, or with a less marked, initial hypertensive phase, and have little or no effect on the BAB (Camras and Bito, 1981; Stern and Bito, 1982). This has prompted the suggestion that PGs may prove useful in the treatment of glaucoma (Bito, 1984a), and a recent study, showed that

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$PGF_{2\alpha}$-isopropylester lowered the IOP in glaucoma patients (Villumsen and Alm, 1987).

The mechanism behind the ocular hypotensive effect of PGs has remained obscure, however. The outflow facility seems to be either unaffected (Crawford, Kaufman and True Gabelt, 1987; Kaufman, 1986) or slightly increased by PGs, but not enough to account for the IOP-reduction (Lee et al., 1984). Nor do PGs diminish the aqueous humor formation (Lee et al., 1984; Camras, Podos, Rosenthal, Lee and Severin, 1987), on the contrary PGs may in some instances even increase the rate of aqueous humor formation (Crawford et al., 1987). This has led to the suggestion that PGs may lower the IOP by increasing the uveoscleral outflow (Bito, 1984a). Indirect evidence supporting this hypothesis has been presented (Crawford and Kaufman, 1987); pilocarpine, which is known to stop the uveoscleral outflow almost completely, prevented the hypotensive effect of $PGF_{2\alpha}$.

The present investigation, in which the effects of a single dose of prostaglandin $F_{2\alpha}$-1-isopropylester ($PGF_{2\alpha}$-IE) on the IOP, aqueous humor flow (AHF), conventional and uveoscleral outflow were studied in cynomolgus monkeys, shows that PGs can indeed increase the uveoscleral outflow. The results show also that this effect is modified by both pilocarpine and atropine. Preliminary reports on parts of this investigation have been presented elsewhere (Nilsson, Stjernschantz and Bill, 1987; Nilsson, Sperber and Bill, 1989).

2. Materials and Methods

27 cynomolgus monkeys (16 female and 11 male), weighing 1·9–3·9 kg, were used for the experiments. Anesthesia was induced by i.m. injection of methohexital sodium (Brietal, Eli–Lilly Co., Indianapolis, IN), 30–50 mg (kg body weight)$^{-1}$, and maintained by i.v. infusion of pentobarbital sodium (Mebumal, ACO, Sweden), 0·3–0·6 mg min^{-1}, through a tail vein. A polyethylene tube, connected to a pressure transducer, was inserted into the tail artery for registration of the arterial blood pressure and collection of blood samples. Heating pads were used to maintain the animals' normal body temperature. Afterwards, the monkeys were allowed to recover, but none was used more than once for this kind of experiment.

Determination of aqueous humor flow, conventional and uveoscleral outflow

The aqueous humor flow was determined by the labeled albumin dilution method described by Sperber and Bill (1984). Three steel cannulas (external diameter of the tip 0·3–0·5 mm) were inserted into the anterior chamber with the help of a special device. Indomethacin, 3 mg (kg body weight)$^{-1}$, was given intravenously before the cannulation of the anterior chamber and about 2–2·5 hr later. One of the cannulas was connected to a pressure transducer for registration of the intraocular pressure. The other two cannulas were used to perfuse the anterior chamber with mock aqueous humor (Sperber and Bill, 1984), containing radio actively labeled albumin. Albumin labeled with [^{125}I] was used in one eye and [^{131}I]-labeled albumin in the other, enabling the AHF to be determined in both eyes simultaneously. An external pump was used to mix the contents of the anterior chamber with the perfusion fluid, and circulate it through a gamma-detector. Thus, the radioactivity in the anterior chamber was continuously analysed and the data were entered into a computer for calculation of the AHF. The data on the radioactivity and the AHF were transferred to a printer and a weighed mean of the three latest values on the AHF was plotted as a function of time on a graphic terminal.

At least during the first hours of the experiments, all the radioactivity appearing in plasma can be assumed to have left the eye via the canal of Schlemm (see Bill, 1984). Determination of the plasma radioactivity can therefore be used to calculate the outflow via the conventional route. The uveoscleral outflow can then be calculated as the difference between the measured AHF and the conventional outflow (Sperber and Bill, 1984). In the

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present experiments, arterial blood samples were collected at 20 min intervals. The flow of anterior chamber fluid to blood (F_B) was calculated according to the following formula:

$$F_B = \frac{42BW(C_2 - C_1)}{C_a t}$$

where 42 is the plasma equivalent albumin space in ml (kg body weight)$^{-1}$, as previously determined (see Sperber and Bill, 1984), BW the body weight in kg, C_1 and C_2 the radioactivity in cpm (ml plasma)$^{-1}$ at the beginning and end of the period respectively, C_a the mean radioactivity in cpm μl^{-1} in the anterior chamber during the period and t the length of the period in min.

Experimental protocols

Prostaglandin $F_{2\alpha}$-1-isopropylester ($PGF_{2\alpha}$-IE), dissolved in saline with 0·5% polysorbate, was used in all experiments. Esterification of the carboxyl group in $PGF_{2\alpha}$ has been shown to increase the penetration into the anterior chamber (Bito and Baroody, 1987), and the ocular hypotensive potency of $PGF_{2\alpha}$ (Bito, 1984b).

In the first series of experiments ($n = 10$), 1 μg free acid equivalents of $PGF_{2\alpha}$-IE in 10 μl was topically applied to one eye in the middle of the first 20 min period, while the contralateral eye received the same amount of vehicle. In the second series of experiments ($n = 8$), the same dose was applied to both eyes in the middle of the first 20 min period. At 80 min, when IOP was expected to begin to decrease, pilocarpine, 10 μg in 5 μl saline, was topically applied to one eye, while the 5 μl saline was applied to the other. In the third series of experiments ($n = 9$), atropine, 1 mg (kg body weight)$^{-1}$, was given intravenously after the cannulation of the anterior chamber, but before the start of the experiment. $PGF_{2\alpha}$-IE and vehicle were then applied as in the first series of experiments.

Statistical analysis was performed by the two-tailed Student's t-test for paired data. All values are given as the mean ± S.E.M. unless otherwise stated. P-values less than 0·05 were considered as significant.

3. Results

The mean arterial blood pressure was not significantly changed in any of the three experimental series [see Figs 1(a), 2(a) and 3(a)]. The results from the experiments with unilateral application of $PGF_{2\alpha}$-IE only are summarized in Fig. 1. In the experimental eye, there was a small, but not statistically significant, initial increase in IOP followed by a slower decrease [(see Fig. 1(b)]. The maximal difference in IOP between the control and the experimental eye was 2·9 ± 0·6 mmHg ($P < 0·001$) and appeared about 3 hr after the application of $PGF_{2\alpha}$-IE.

The aqueous humor flow increased on the $PGF_{2\alpha}$-IE-treated side during the first 1–1·5 hr and then slowly declined through the rest of the experiment [see Fig. 1(c)]. The mean AHF during the whole experiment (4 hr) was slightly higher for the experimental than for the control eye; 1·37 ± 0·11 compared with 1·16 ± 0·09 μl min^{-1}, difference 0·18 ± 0·06 μl min^{-1} ($P < 0·05$). The flow of aqueous humor to the blood, corresponding to the conventional outflow, was lower on the experimental side throughout [see Fig. 1(d)]. The mean values for the control and the experimental side were 0·55 ± 0·07 and 0·36 ± 0·04 μl min^{-1}, difference 0·19 ± 0·04 μl min^{-1} ($P < 0·01$).

The uveoscleral outflow began to increase in the experimental eye already during the first hour and was maximal 1·5–2 hr after the application of $PGF_{2\alpha}$-IE. During the second half of the experiments, the uveoscleral outflow tended to increase also in the control eye [see Fig. 1(e)]. The mean uveoscleral outflow was 0·98 ± 0·12 μl min^{-1} for the experimental, and 0·61 ± 0·10 μl min^{-1} for the control eye, difference 0·37 ± 0·04 μl min^{-1} ($P < 0·001$).

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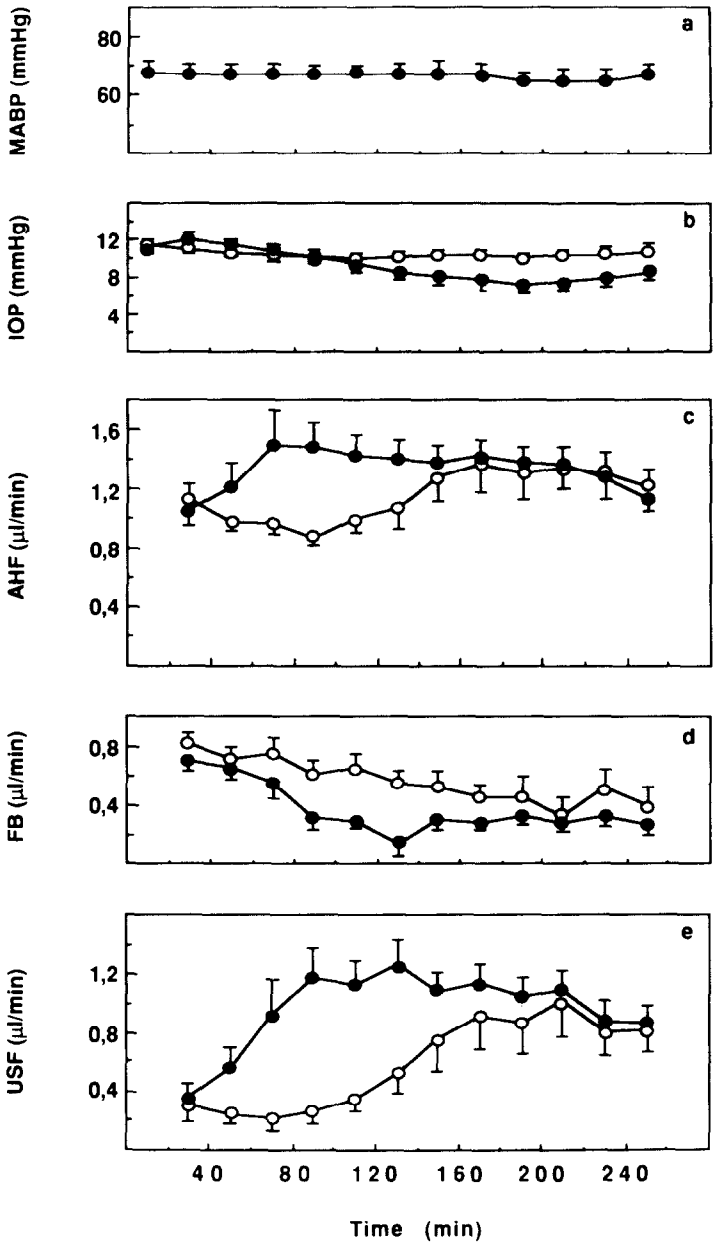

FIG. 1. The effects of unilateral topical application of prostaglandin $F_{2\alpha}$-1-isopropylester ($PGF_{2\alpha}$-IE) on (a) the mean arterial blood pressure (MABP), (b) intraocular pressure (IOP), (c) aqueous humor flow (AHF), (d) flow of aqueous humor to the blood (FB) and (e) uveoscleral outflow (USF). Each point represents the mean value during a 20 min period. The application of $PGF_{2\alpha}$-IE, 1 μg free acid equivalents in 10 μl, to the experimental eye (●) and 10 μl vehicle to the control eye (○) was made in the middle of the first 20 min period. Mean values and S.E.M. are given ($n = 10$).

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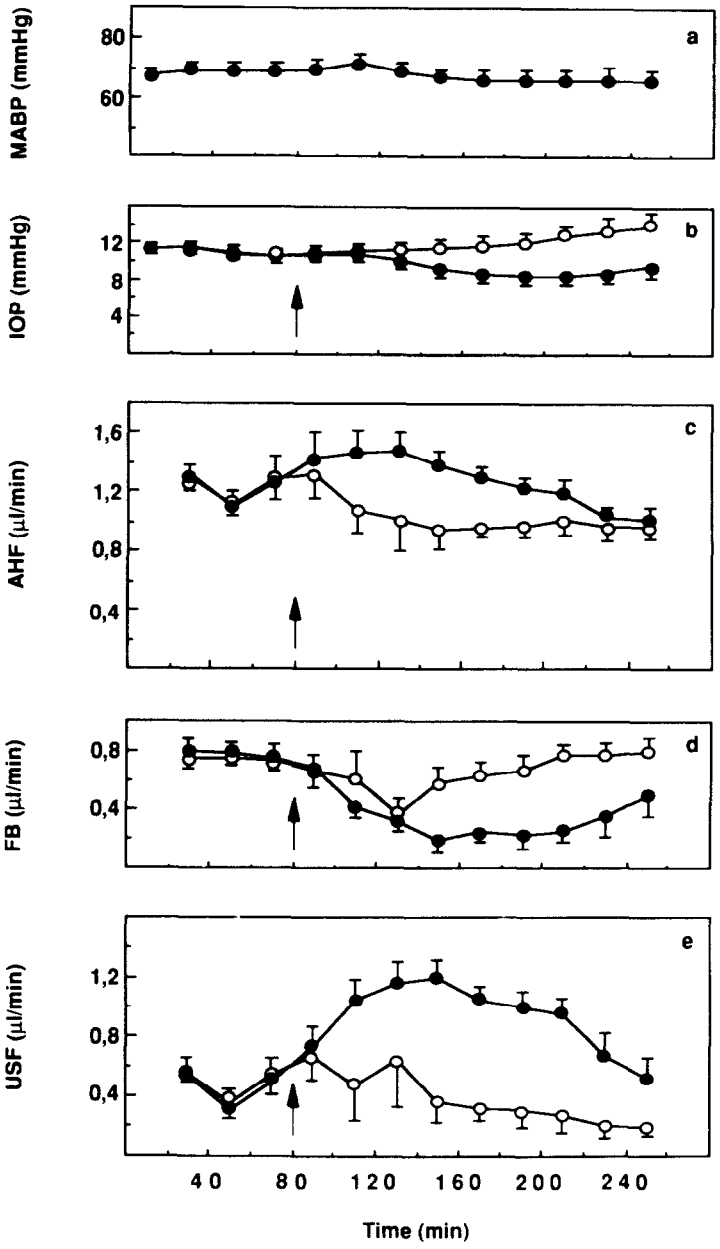

FIG. 2. The effects of unilateral topical application of pilocarpine after the bilateral topical application of prostaglandin $F_{2\alpha}$-1-isopropylester ($PGF_{2\alpha}$-IE) on (a) the mean arterial blood pressure (MABP), (b) intraocular pressure (IOP), (c) aqueous humor flow (AHF), (d) flow of aqueous humor to the blood (FB) and (e) uveoscleral outflow (USF). Each point represents the mean value during a 20 min period. $PGF_{2\alpha}$-IE 1 µg free acid equivalents in 10 µl, was applied bilaterally in the middle of the first 20 min period. At 80 min, indicated by the arrow, pilocarpine, 10 µg in 5 µl, was topically applied to one eye. $PGF_{2\alpha}$-IE (●). $PGF_{2\alpha}$-IE + pilocarpine (○). Mean values and S.E.M. are given ($n = 8$).

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