Metabolism of Arachidonic Acid in Rabbit Iris and Retina

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Both the iris and the retina of the rabbit released prostaglandins (PG) E₂, F₂α, 6-keto-F₂α and thromboxane (Tx) B₂, when incubated in vitro. PGE₂ was the major cyclooxygenase product formed by each tissue. The kinetics of PGE₂ release by the iris and the retina were similar: high initial output followed by a decline to a steady-state value. The production of PGE₂ was inhibited by indomethacin and stimulated by ionophore A23187. The iris and the retina converted exogenous arachidonic acid into 12- and 15-hydroxy-eicosatetraenoic acid (HETE): inhibition by eicosatetraynoic acid (ETYA) indicated the involvement of lipoxygenase enzymes. This lipoxygenase activity was important relatively to cyclooxygenase in the retina, but was only a minor pathway in the iris. Leukotriene (LT) B₄ was released by the iris and the retina in amounts smaller than PGE₂, but compatible with a biological activity: ionophore A23187 stimulated LT B₄ production in both tissues. Our data support the hypothesis that PGE₂ and LT B₄ could play a role in the initiation of ocular inflammation. Invest Ophthalmol Vis Sci 26:1336-1342, 1985

Prostaglandins (PG) exert multiple pharmacologic actions on the eye. Their intracameral or topical application increases the intraocular pressure and the protein content of the aqueous humor in the rabbit, as a consequence of vasodilation and increased vascular permeability in the iris. Prostaglandins stimulate contraction of the iris sphincter muscle and trigger miosis. An inhibitory effect of prostaglandins on the electrical activity of the rabbit retina has been reported. PGE₁ and PGE₂ induce the neovascularization of the rabbit cornea. Eicosanoids other than prostaglandins are also active: leukotriene (LT) B₄ induces the entry of polymorphonuclear leukocytes in the aqueous humor; LTC₄ and LTD₄ constrict the pupil and decrease the iris blood flow in the cat. It is known for a long time that the eye has the capacity to produce some prostaglandins. Ambache initially reported the presence in rabbit iris of a smooth muscle stimulating activity that he called irin: one component of irin was later identified as PGF₂α. Prostaglandin-like activity has been detected in the aqueous humor after mechanical trauma or during acute inflammatory in rabbit and man. Subsequent studies have investigated the transformation of exogenous radiolabeled arachidonic acid by various ocular preparations. We have now characterized and quantitated the production of PGs and Tx B₂ from endogenous arachidonic acid and investigated the existence of a lipoxygenase pathway, in the intact retina and iris from the rabbit, the animal most frequently used in ophthalmologic research.

Materials and Methods

Materials

Prostaglandin 6-keto-F₁₅ (6-K-PGF₁₅), PGE₂, PGF₂α, and thromboxane (Tx) B₂ were purchased from Upjohn Diagnostics (Kalamazoo, MI). 3H-labeled tracers of arachidonic acid, PGE₂, PGF₂α, 6-K-PGF₁₅ and Tx B₂ and the LT B₄ assay kit were obtained from Amersham (Amersham, England). Antisera against PGE₂ and PGF₂α were purchased respectively from Institut Pasteur de Paris and Clinical Assays (Cambridge, MA). Antisera against 6-K-PGF₁₅ and Tx B₂ were generously given by Dr. J. Beetens (Universitaire Instelling Antwerpen, Antwerp) and Dr. J. B. Smith (Cardeza Foundation; Philadelphia, PA), respectively. Optifluor scintillation liquid, organic solvents, and ionophore A23187 were purchased respectively from Packard Instrument, Burdick-Jackson (Burdick & Jackson Laboratories, Inc., Muskegon, MI), and Boehringer Mannheim (Mannheim, West Germany). Eicosatetraynoic acid (ETYA) was a generous gift of Dr. J. G. Hamilton (Hoffman-La Roche; Nutley, NJ) and in-

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This work was performed under contract of the Ministère de la Politique Scientifique ("Actions Concertées") and was supported by a grant from Merck-Sharp-Dohme. D. Demolle was supported by ARBD and APMO.

Submitted for publication: August 28, 1984.

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domethacin was received from Merck-Sharp-Dohme (West Point, PA).

**Incubation of Iris and Retina**

White rabbits weighing about 3 kg were killed by a blow on the neck, in conformity to the ARVO Resolution on the Use of Animals in Research. The eyes were removed and the iris and the retina were rapidly dissected. They were incubated at 37°C under constant shaking (80 rpm) in a medium of the following composition: NaCl, 124 mM; KCl, 5 mM; MgSO4, 1.25 mM; CaCl2, 1.45 mM; KH2PO4, 1.25 mM; Hepes buffer pH 7.4, 25 mM; glucose, 8 mM. Usually, the iris and the retina were incubated for five periods of 30 min in 2 ml medium; the medium was renewed at the end of each period. Stimuli (ionophore A23187, 50 μM; domethacin, 10 μM) were added to the incubation medium at the beginning of the last period. Results were expressed as ng per mg tissue in the case of the iris or as ng per whole retina. Histologic examinations indicated that the preparations used were free from contaminating tissues.

**Radioimmunoassay of PGs, TXB2, and LTB4**

PGE2, PGF2α, 6-K-PGF1α, and TXB2 released in the incubation medium were measured directly by radioimmunoassay (RIA), as described.30 LTB4 was measured by a similar procedure, except for the use of a Tris buffer, pH 8.6, rather than pH 7.4 and an overnight incubation at 4°C rather than a 1-hr incubation at room temperature. In some experiments, RIA were performed after purification of the incubation media by reversed-phase-high-performance liquid chromatography (RP-HPLC), using a μ Bondapak C18 column (3.9 × 300 mm, 10 μ particles: Waters Associates; Milford, MA).30

**Detection of Hydroxyeicosatetraenoic Acids (HETEs)**

After the addition of 17-hydroxydocosahexaenoic acid-methyl ester, as internal standard, samples of iris and retina incubation media were extracted with ethyl acetate. After evaporation under nitrogen, the dry residue of the extract was dissolved in 50 μl methanol and injected on a Nucleosil C18 column (4 × 250 mm, 5 μ particles: Macherey-Nagel; Durer, West Germany). Elution was performed with methanol-water-acetic acid (75:25:0.01, v/v) at a flow rate of 1 ml/min and the UV absorbance at 234 nm was monitored.

In other experiments, the iris and the retina were incubated for five periods of 30 min in 2 ml medium, as described above. At the beginning of the last period, [3H]-arachidonic acid (0.5 μCi/ml; 0.5 μg/ml) purified by RP-HPLC just prior to the experiment, was added. At the end of the incubation, the tissue was removed and a mixture of 5-, 12-, and 15-hydroxy-eicosatetraenoic acid (HETE) was added to the incubation medium, which was then extracted with ethyl acetate, at neutral pH. After evaporation under nitrogen, the dry residue of the extract was dissolved in 50 μl methanol and injected on a Biosil-ODS-SS column (4 × 250 mm, 5 μ spherical particles; Biorad), which was then eluted with a mixture of methanol-water-acetic acid, first in the ratio 75:25:0.01 (v/v) for 20 min and then in the ratio 86:14:0.01 (v/v) for 15 min (flow rate = 1.5 ml/min). UV absorbance at 234 nm was monitored (using a variable wavelength LC-UV detector, Pye Unicam) and 0.6-ml fractions were collected for liquid scintillation counting. 5-HETE was obtained by chemical synthesis; 12-HETE was biosynthesized using washed human platelets; 15-HETE and 17-hydroxydocosahexaenoic acid were generated by reaction of arachidonic acid and docosahexaenoic acid, respectively, with soybean lipoxidase.

**Results**

In order to characterize the profile of endogenous cyclooxygenase products released by the iris and the retina of the rabbit, samples of incubation media were submitted to RP-HPLC and 6-K-PGF1α, TXB2, PGF2α, and PGE2 were measured by RIA in the fractions collected (Figs. 1, 2). The major product released by the retina was PGE2 followed by 6-K-PGF1α; smaller amounts of TXB2 and PGF2α were also detected (Fig. 1). In the iris, the predominance of PGE2 was even more striking: PGF2α, 6-K-PGF1α, and TXB2 were also produced, in a decreasing order of quantities (Fig. 2). The kinetics of PGE2 release were similar in the iris and the retina (Figs. 3, 4). The release was maximal during the first 30-min incubation period and then declined to reach a steady state after 30 or 60 min. During the first 30 min, the amounts of PGE2 released were: 163 ± 33 ng per iris (mean ± SE, n = 8)—representing a mean value of 2.9 ng/mg tissue—and 13 ± 3 ng per retina (mean ± SE, n = 6). The mean steady-state values were 45 ng per iris and 2 ng per retina. Indomethacin (28 μM) inhibited the release of PGE2 (Figs. 3, 4). In the iris, a 54% inhibition was observed during the first 30 min and an 89% inhibition was obtained between 60 and 90 min (mean of four experiments). In the retina, the corresponding rates of inhibition were 50% and 78%, respectively (mean of three experiments). The production of PGE2 was stimulated by ionophore A23187 (Figs. 3, 4). The amounts of PGE2 released during a 30-min incubation in the presence of A23187 (10 μM) were 389% (mean of four experiments) and 635% (mean of three experiments) of the amounts released during the preceding 30-min incubation period, used as control, respectively in the iris and the retina.
A23187 also stimulated the release of the other cyclooxygenase products (Fig. 2). The addition of exogenous arachidonic acid increased the release of the 4 cyclooxygenase products measured (not shown).

Attempts to demonstrate the release of one of the HETEs by the iris or the retina of the rabbit, either spontaneously or under stimulation by ionophore A23187, were unsuccessful; no peak having a retention time corresponding to those of HETEs could be detected, using RP-HPLC with UV detection at 234 min. Therefore, the transformation of exogenous radiolabeled arachidonic acid was studied. RP-HPLC analysis of the incubation media, collected after a 30-min exposure of the retina or the iris to [3H]-arachidonic acid, revealed two radioactive peaks co-eluting with 15-HETE and 12-HETE standards (Fig. 5). No peak co-eluting with 5-HETE was detected. In the retina, 1% of the added arachidonic acid was converted into 12-HETE and 0.3% in 15-HETE (mean of four experiments). ETYA (50 μM) inhibited the formation of 12-HETE by 65% and that of 15-HETE by 40%. In the iris, the rates of conversion were 0.33% for 12-HETE and 0.25% for 15-HETE (mean of four experiments). Inhibition by ETYA was less striking: 38% for 12-HETE and 36% for 15-HETE. By comparison, the rates of conversion of exogenous [3H]-arachidonic acid into PGE2 (determined by a similar RP-HPLC analysis) were 2.5% in the iris and 0.35% for the retina.

The release of LTB4 from the iris and the retina was detected by RIA (Fig. 6). During the initial 30-min incubation, the amounts of LTB4 released were: 0.35 ± 0.15 ng per iris (mean ± SD, n = 4)—representing a mean value of 2.9 pg/mg tissue—and 0.7 ± 0.3 ng per retina (mean ± SD, n = 4). Ionophore A23187 stimulated the release of LTB4 (Fig. 6). The amounts of LTB4 released during a 30-min incubation in the
presence of A23187 (10 μM) were 1045% and 400% of the amounts released during the preceding 30-min period, used as control, respectively in the iris and the retina (mean of two experiments).

Discussion

The metabolism of arachidonic acid in the eye has been studied so far using two different experimental approaches. In initial studies, prostaglandin-like activity was detected in the iris and in the aqueous humor after mechanical stimulation or during acute uveitis, using bioassay coupled to thin-layer chromatography. In further studies, the transformation of exogenous radiolabeled substrate was investigated in various ocular preparations. Van Dorp et al showed that a pig iris particulate fraction converted eicosatrienoic acid into PGF₁ and PGF₁α, whereas no activity was found in the retina. Acellular preparations of rabbit iris converted [14C]-arachidonic acid into a mixture of PGs and TxB₂; the relative amounts of these various products were critically dependent on the experimental conditions (in particular, the concentrations of epinephrine and glutathione). Arachidonic acid was also metabolized by the rabbit conjunctiva, whereas the retina was inactive. Microsomes of bovine iris transformed the endoperoxide PGH₂ into prostacyclin but not TxA₂; in human iris, both products were formed. It was recently shown that bovine and rat retina transformed arachidonic acid into almost equal amounts of 6-K-PGF₁α, PGE₂, PGF₂α, and TxB₂; however, the main compounds formed from exogenous
In the first part of this study, we have measured the formation of cyclooxygenase products from endogenous arachidonic acid in intact iris and retina of the rabbit, which constitutes a classical experimental model in ophthalmology. The results are thus consistent with several recent studies, which have shown the presence of C12 lipoxygenase in bovine and rat retina \(^{25,26}\) and in the iris of various species, including rabbit and man. \(^{27-29}\) At variance with these authors, \(^{25,27-29}\) we were unable to detect the conversion of arachidonic acid into 5-HETE by the rabbit iris and retina. It might be that, under our experimental conditions, this compound was esterified into tissue phospholipids \(^{26}\) or that, as described for neutrophils, \(^{31}\) its generation from exogenous arachidonic acid requires an additional stimulus like ionophore A23187. We have however observed the release of LTC4 from rabbit iris and retina and its stimulation by ionophore A23187. Relatively to PGE2, LTC4 was a minor product, especially in the iris. Nevertheless, the concentrations of LTC4 reached in the incubation fluid of the iris (±0.7 ng/ml) and the retina (±0.6 ng/ml) were within the range in which LTC4 exerts its chemotactic effect on polymorphonuclear leukocytes, ie, 10 pg/ml to 5 ng/ml. \(^{32}\)

Whereas the possible action of 12-HETE in the retina remains unclear, the pharmacologic effects of PGE2 on the eye are well known. PGE2 reproduces most features of vascular inflammation (vasodilation and increased vascular permeability of the iris, protein exudation in the aqueous humor, increased intraocular pressure) \(^{1-7}\); it also exerts a vasculogenic activity. \(^{12}\) Our observations that PGE2 is the main cyclooxygenase product formed in both iris and retina and that it is spontaneously re-
leased from these tissues following mechanical manipulation support the hypothesis that PGE2 could play some role in the initiation of ocular inflammation and possibly neovascularization. In addition, LTB4 might be responsible for another aspect of ocular inflammation, the infiltration of leukocytes.

Key words: arachidonic acid, prostaglandin, thromboxane, lipooxygenase, leukotriene, iris, retina, rabbit

Acknowledgments

The authors thank Mrs. N. Galand for her excellent technical assistance and Mrs. Gh. Wilmes for typing the manuscript. The authors are grateful to Dr. A. Zanen for his interest in this work.

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