Leukotriene Modulation of Chloride Transport in Frog Cornea

Barry E. Schoeffer and Jose A. Zadunaisky

The present study has identified the metabolites of arachidonic acid (AA) formed by the isolated frog cornea and has shown that this tissue is capable of the biosynthesis of both lipoygenase and cyclooxygenase pathway metabolites. The cyclooxygenase (CO) metabolites found in greatest abundance were the prostaglandins E₂ and F₂α (PGE₂ and PGF₂α). The major lipoygenase (LO) pathway metabolite found by thin-layer chromatography (TLC) was leukotriene B₄ (LTB₄), whereas leukotriene C₄ (LTC₄) biosynthesis was detected by radioimmunoassay. These leukotrienes (LTs) are highly potent modulators of active chloride transport, since incubation with LTC₄ (10⁻¹⁷-10⁻⁹ M) resulted in a dose-dependent stimulation of both the Cl⁻ originated short-circuit current (SCC) and potential difference (PD). In contrast, LTB₄ (10⁻⁷-10⁻⁹ M) inhibited both of these parameters. Both LTC₄ and LTB₄ exerted these effects only when applied to the endothelial side. Preincubation with the leukotriene receptor antagonist, FPL 55712 completely blocked the response to LTC₄, indicating that the action of LTC₄ in the cornea is receptor-mediated. In this report the authors show that LTB₄ and LTC₄ are formed by the cornea and that they are potent modulators of the SCC and PD in chamber experiments. The possibility exists that LTB₄ and LTC₄ may function as endogenous regulators of net Cl⁻ transport in the cornea, since the addition of these metabolites resulted in a dose-dependent stimulation (with LTC₄), and inhibition (with LTB₄), of both the short-circuit current (SCC) and potential difference (PD). Invest Ophthalmol Vis Sci 27:898-904, 1986

We have previously shown that the application of arachidonic acid (AA) to isolated frog corneas resulted in a large stimulation of net chloride transport. In this tissue, AA is metabolized via an active cyclooxygenase (CO) pathway to form primarily prostaglandin E₂ (PGE₂), which stimulates adenylylate cyclase activity in a dose-dependent fashion and increases cyclic AMP (cAMP) levels. The stimulatory effect of AA on net Cl⁻ transport appears to result from an increase in cAMP content since beta agonists increase cAMP levels and stimulate net Cl⁻ transport. This conclusion is further supported by the observation that pre-treatment of corneas with the CO inhibitor, indomethacin, blocked the stimulatory effect of AA on both PGE₂ and cAMP synthesis. Nevertheless, in those indomethacin pre-treated corneas, AA still elicited a significant, although smaller, increase in short-circuit current (SCC) following addition of exogenous AA. This result suggested that AA or one its metabolites may stimulate chloride transport by an additional mechanism. We therefore began to test the ability of a variety of lipoygenase pathway metabolites of AA to alter Cl⁻ transport.

Metabolite formation from AA proceeds primarily via two enzymatic pathways: the cyclooxygenase (CO) and lipoygenase (LO) pathways. Both of these pathways comprise the two branches of the "AA cascade," and the metabolites formed are known as eicosanoids. The CO branch results in the formation of prostaglandins, thromboxane, and prostacyclin. The more recently discovered LO branch has been shown to give rise to a novel series of highly potent eicosanoids. Most notable among these are the leukotrienes (LTs). It is now becoming clear that many different cell types have lipoygenase activity and that these potent compounds can regulate diverse physiological functions in addition to their ability to mediate inflammation. In view of the rapidly expanding knowledge of the lipoygenase pathway, and particularly the biological activities of the leukotrienes, this study was initiated in order to investigate the effects of lipoygenase pathway metabolites on active chloride transport in the cornea. It was also of interest to determine whether an active lipox-
ygenase pathway exists in the cornea and to identify those eicosanoids formed by the cornea.

**Materials and Methods**

Corneas were dissected from active, nonhibernating bull frogs (*Rana catesbeiana*) and then either briefly homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) or left intact. Incubations were carried out in 0.5 ml amphibian Ringer's solution\(^8\) containing glucose (90 mg/100 ml). Each sample contained two corneas or homogenate from two corneas, which was incubated for 1 hr at 24°C with gentle stirring, in the presence of \(^14\)C(U)-AA (0.25 \(\mu\)Ci, specific activity, 390 mCi/mmol (New England Nuclear, Boston, MA). All procedures adhered to the ARVO Resolution on the Use of Animals in Research.

**Thin Layer Chromatography (TLC)**

Following incubation, samples were acidified to pH 3.0 with citric acid, corneal tissue was removed (except when homogenates were used), and the remaining Ringer's solution or homogenate was extracted twice with five volumes of chloroform.\(^9\) Extracts were evaporated to dryness under a stream of nitrogen gas, and re-dissolved in 50 \(\mu\)l of chloroform-methanol (2:1). Samples were then spotted onto silica gel G Chromatograph sheets (Eastman Kodak; Rochester, NY) under nitrogen, along with standards of authentic PGs, LTs, and HETEs.

Metabolite identifications were based on the co-migration of authentic standards with radioactive bands in at least two different solvent systems. A total of four different solvent systems were used.\(^10\) Solvent system A (see Fig. 1A), consisting of the organic phase of iso-octane:ethyl acetate:acetic acid: \(\text{H}_2\text{O} (30:66:12:60)\) was routinely used because it alone was capable of clearly separating each AA metabolite of interest. Other solvent systems used for identification of metabolites were: solvent system B, (Fig. 1B), benzene:dioxane:acetic acid (20:10:1); solvent system C, (Fig. 1C), chloroform: methanol:water:acetic acid (90:9:0.65:1); solvent system D, (Fig. 1D), benzene:diethyl ether:ethanol:acetic acid (50:40:2:0.2). None of these additional systems can separate each metabolite. However, the use of the four solvent systems shown in Figure 1 (upper) permits the identification of most metabolites at least twice.

To visualize metabolites and labeled standards, the dried chromatograph sheets were sprayed with an autoradiography enhancer (New England Nuclear) and then exposed to Kodak DEF-5 X-Ray film for 72–96 hr. Unlabeled standards were visualized by placing the chromatography sheet briefly in iodine vapor or by using fluorescent chromatograph sheets.

In order to rule out the possibility of metabolite formation via auto-oxidation of AA, \(^14\)C(U)-AA was incubated with corneas that were first boiled for 30 min (Fig. 1H). In addition, blanks were incubated containing only radiolabeled AA and no tissue (Fig. 1I). In both of these cases, as can be seen, no radioactive bands were observed other than free AA at the top of the TLC autoradiograph, indicating that metabolites are produced only via enzymatic processes, not via auto-oxidation. Radiolabeled AA, LTs, and PGs were spotted directly onto TLC plates to verify that no contaminants or oxidation products were present. Each of these were found to be composed of only a single species. To avoid oxidation, LTs were divided into batches before storage at \(-70°C\) under \(\text{N}_2\) gas.

**Determination of Leukotriene C\(_4\) and Prostaglandin E\(_2\) Biosynthesis by Radioimmunoassay**

Radioimmunoassay of LTC\(_4\) was performed on unextracted Ringer's samples following the incubation of whole frog corneas in Ringer's for 1 hr with or without AA (3 \(\mu\)M) and calcium ionophore A23187 (2 \(\mu\)M). The assay was performed using the New England Nuclear Leukotriene C\(_4\) (\(^1\)H)-RIA Kit (NEK-030). The assay consisted of 10 control samples in which corneas were only incubated in Ringer's, 20 samples containing corneas incubated with Ringer's and unlabeled AA, and 16 samples containing corneas incubated with AA plus calcium ionophore A23187 in Ringer's. Each sample was assayed in duplicate.

Radioimmunoassay of prostaglandin E\(_2\) (PGE\(_2\)) was similarly performed on unextracted Ringer's samples following incubation of whole corneas in Ringer's. Corneas were pre-incubated in Ringer's with 3 \(\mu\)M AA for 1 hr and then rinsed three times to remove free AA. Corneas were then incubated with slight agitation in Ringer's for 0.5 hr. After incubation, the corneas were removed, and the incubation medium was utilized for the assay. The assay was performed using the Amersham (Arlington Heights, IL) RIA kit for bicyclic PGE\(_2\) (TRK 800). Seven samples were run, with each sample containing two corneas incubated in 0.5 ml Ringer's. Each sample was split and assayed in duplicate.

**Measurement of Chloride Transport**

The isolated frog cornea is well established as a convenient model system for the study of chloride transport.\(^5\) Chloride ions are transported from the aqueous to tear side by a chloride pump located in the epithelium.\(^11\)\(^12\) In order to study the effects of AA metabolites on chloride transport, freshly dissected corneas were mounted in an Ussing-type chamber, with either NaCl or Na\(_2\)SO\(_4\) Ringer's solution bathing corneal surfaces as previously described.\(^1\) The short-circuit current...
Fig. 1 A–D (Upper) shows TLC autoradiographs of corneal homogenates incubated with $^{14}$C(U)-AA and run in four different solvent systems to facilitate identification of metabolite bands as described in Materials and Methods. A, shows corneal homogenate extracts run in solvent A, consisting of iso-octane:ethyl acetate:acetic acid:water. E–I (Lower): TLC autoradiographs run with solvent system A (A above), showing E—whole unhomogenized cornea, F—corneal endothelium, G—corneal epithelium, H—boiled cornea, and I—$^{14}$C(U)AA blank incubated without tissue.
(SCC), which is nearly proportional to the net Cl⁻ transport across the cornea, and potential difference (PD), were measured with dual automatic voltage clamp units. Eicosanoids to be tested were added directly to the Ringer’s solution as 2 μl aliquots to either one or both chamber sides, with dilution occurring rapidly in the chamber with the aid of bubble-lift circulation of the Ringer’s solution. LTC₄, LTD₄ and LTE₄ were dissolved in distilled water. LTB₄ and 5-HETE were dissolved in methanol. Appropriate controls were carried out showing no effect of these solvents on the SCC or the PD.

*Rana catesbeiana* were obtained from a local supplier and were kept in running tap water until used. Synthetic prostaglandins, thromboxane and AA for standards were from Sigma (St. Louis, MO). Tritiated LTB₄ and LTC₄ for standards were from Amersham. Tritiated LTD₄, 5-HETE, 12-HETE, and 15-HETE (hydroxy-eicosatetraenoic acid) for standards and ¹⁴C(A)-AA were from New England Nuclear. The synthetic leukotrienes LTB₄, LTC₄, LTD₄, LTE₄, and 5-HETE were a gift from Dr. J. Rokach (Merck-Frosst Canada, Inc.). FPL-55712 was obtained from Fisons (UK).

**Results**

**AA Metabolites Formed by Cornea**

*Corneal homogenates and whole cornea*: Figure 1 shows metabolites of AA that were isolated and identified by TLC-autoradiography. The autoradiographs indicate that PGE₂ is by far the major eicosanoid formed, with LTB₄ and PGF₂α the second and third most abundant, respectively. Six cyclo-oxygenase products have been identified in frog cornea: PGE₂, PGF₂α, PGD₂, thromboxane (identified as the TXB₂ degradation product), PGA₂, and prostacyclin (identified as the 6-keto PGF₁α degradation product). We have also identified three lipoxygenase derivatives by this method: LTB₄, 5-HETE and 12-HETE, and their presence indicates that frog cornea contains at least two active lipoxygenases specific for the 5- and 12-position oxidation of arachidonate. In addition, other bands were also observed in the autoradiographs, but these have not yet been identified.

A comparison between incubations with homogenates and whole corneas (Fig. 1A and E) reveals that many of the same metabolites are formed in each case, with similar percent distributions. This indicates that viable, intact corneas are capable of synthesizing the same AA metabolites synthesized by homogenates.

It should be noted that in these experiments, where the tissue is incubated with exogenous ¹⁴C-AA, the information obtained indicates which AA metabolites the tissue is capable of synthesizing. This method tells us little about endogenous levels of AA metabolite synthesis under normal conditions because the addition of ¹⁴C-AA may result in the stimulated synthesis of metabolites.

**LTC₄ biosynthesis**: Determination of LTC₄ biosynthesis by radioimmunoassay was done because the TLC method cannot isolate LTC₄. RIA measurements revealed that frog corneas have the biosynthetic capacity to produce LTC₄ when incubated with AA substrate and calcium ionophore A23187. Samples incubated without calcium ionophore A23187 resulted in no detectable LTC₄ synthesis. In samples incubated with calcium ionophore and AA the rate of LTC₄ formation was 0.668 ± 0.021 ng LTC₄/cornea/hour (n = 16).

**PGE₂ biosynthesis**: Determination of PGE₂ biosynthesis by radioimmunoassay was done to verify and quantitate PGE₂ formation, since the PGE₂ band visualized in TLC-autoradiographs appears to be the predominant cyclo-oxygenase product. RIA measurements confirmed PGE₂ formation at the rate of 5.00 ± 0.46 ng PGE₂/cornea/hr (n = 7).

**Corneal epithelium vs endothelium**: It was of interest to determine which corneal layers were responsible for the biosynthesis of arachidonate metabolites. To that end, experiments were done in which either corneal epithelium or endothelium was removed, and then incubations were carried out as for whole cornea. Epithelium was removed by carefully scraping the corneal surface of the intact eye, and then dissecting off the cornea, yielding intact endothelium attached to stroma. Epithelium-stroma preparations were obtained by cutting half-way around the cornea-scleral junction, resulting in a flap which allowed the insertion of a small rounded spatula for scraping off the endothelium. The cornea was then removed and used in the experiment. Removal of epithelium and endothelium was monitored by the use of a dissecting microscope. Figures 1F and G show that in both endothelium and epithelium synthesis of PGE₂ and LTB₄ predominate, accounting for most of the eicosanoid synthesis in each. This demonstrates that cyclo-oxygenase as well as lipoxygenase activity is present in both of these tissues. Other minor eicosanoid bands are present in roughly similar proportions, suggesting that endothelium and epithelium have similar capacities for eicosanoid synthesis.

**Effects of Eicosanoids on Chloride Transport**

*Leukotriene C₄ (LTC₄)*: In order to study the effects of synthetic leukotrienes on the SCC and PD, corneas were mounted in Ussing-type chambers. LTC₄ was applied to the endothelial side of the chamber, with final concentrations ranging from 10⁻⁷–10⁻⁹ M. Within 3
Table 1. The effects of leukotrienes on the electrical properties of isolated frog cornea in Ussing-type chambers*  

<table>
<thead>
<tr>
<th>Experiment</th>
<th>SCC (µA·cm⁻²)</th>
<th>PD (mV)</th>
<th>R (ohm·cm⁻²)</th>
<th>Time to maximum effect (min)</th>
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<tbody>
<tr>
<td>Control (6)</td>
<td>20.6 ± 1.5</td>
<td>21.3 ± 1.0</td>
<td>1067 ± 89</td>
<td>10</td>
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<tr>
<td>LTC₄ (10⁻⁷ M)</td>
<td>31.4 ± 2.1</td>
<td>26.3 ± 1.2</td>
<td>863 ± 76</td>
<td>10</td>
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<tr>
<td>Change (%)</td>
<td>+52.4 (p &lt; 0.001)</td>
<td>+23.5 (p &lt; 0.001)</td>
<td>-19.1 (p &lt; 0.001)</td>
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<tr>
<td>Control (3)</td>
<td>12.4 ± 0.9</td>
<td>20.8 ± 2.2</td>
<td>1677 ± 161</td>
<td>11</td>
</tr>
<tr>
<td>LTC₄ (10⁻⁸ M)</td>
<td>16.5 ± 0.8</td>
<td>23.9 ± 1.7</td>
<td>1452 ± 87</td>
<td>11</td>
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<tr>
<td>Change (%)</td>
<td>+33.1 (p &lt; 0.005)</td>
<td>+4.9 (NS)</td>
<td>-13.4 (NS)</td>
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<td>Control (3)</td>
<td>11.1 ± 0.9</td>
<td>16.9 ± 2.3</td>
<td>1506 ± 81</td>
<td>12</td>
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<tr>
<td>LTC₄ (10⁻⁹ M)</td>
<td>12.9 ± 1.2</td>
<td>18.3 ± 2.5</td>
<td>1403 ± 54</td>
<td>12</td>
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<tr>
<td>Change (%)</td>
<td>+16.2 (NS)</td>
<td>+8.3 (NS)</td>
<td>-6.8 (NS)</td>
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</tr>
<tr>
<td>Control (6)</td>
<td>21.6 ± 2.8</td>
<td>25.2 ± 2.8</td>
<td>1253 ± 151</td>
<td>15</td>
</tr>
<tr>
<td>LTB₄ (10⁻⁷ M)</td>
<td>10.9 ± 1.4</td>
<td>18.8 ± 2.7</td>
<td>1836 ± 258</td>
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<tr>
<td>Change (%)</td>
<td>-49.5 (p &lt; 0.005)</td>
<td>-25.4 (p &lt; 0.005)</td>
<td>+46.5 (p &lt; 0.01)</td>
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<td>Control (4)</td>
<td>12.3 ± 1.3</td>
<td>20.2 ± 2.3</td>
<td>1644 ± 97</td>
<td>21</td>
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<tr>
<td>LTB₄ (10⁻⁸ M)</td>
<td>7.2 ± 0.7</td>
<td>14.9 ± 1.8</td>
<td>2091 ± 165</td>
<td>21</td>
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<tr>
<td>Change (%)</td>
<td>-41.5 (p &lt; 0.02)</td>
<td>-26.2 (p &lt; 0.005)</td>
<td>+27.2 (NS)</td>
<td></td>
</tr>
<tr>
<td>Control (3)</td>
<td>13.9 ± 2.1</td>
<td>19.2 ± 2.0</td>
<td>1366 ± 146</td>
<td>30</td>
</tr>
<tr>
<td>LTB₄ (10⁻⁹ M)</td>
<td>12.2 ± 1.7</td>
<td>18.3 ± 2.0</td>
<td>1531 ± 136</td>
<td>30</td>
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<tr>
<td>Change (%)</td>
<td>-12.2 (NS)</td>
<td>-4.7 (p &lt; 0.02)</td>
<td>+12.1 (NS)</td>
<td></td>
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</table>

*Results are expressed as the mean ± S.E. Number of experiments is given in parenthesis. Level of significance (p) is based on two-tailed Student's t-test. NS = p > 0.02.

min after exposure to 10⁻⁷ M LTC₄, a highly significant stimulation in both the SCC and PD was observed, along with a decrease in tissue resistance (Table 1 and Fig. 2, upper). Maximal stimulation of the SCC was observed 10 min following the addition of LTC₄. The SCC and PD gradually declined and returned to baseline levels within 1 hr. Repeated applications of LTC₄ (10⁻⁷ M) were entirely without effect, following the initial stimulation, indicating a saturation effect.

The addition of LTC₄ to the epithelial side of the chamber was entirely without effect, even though the subsequent addition of LTC₄ to the endothelial side resulted in a stimulation.

Leukotriene B₄ (LTB₄): Additions of LTB₄ to the endothelial side of the chamber resulted in a dose-dependent decrease in the SCC and PD. The SCC trace in Figure 2 (lower) shows the typical suppression of the SCC and PD with 10⁻⁷ M LTB₄. Table 1 indicates that at 10⁻⁷ M, the mean suppression of SCC and PD as well as the increase in tissue resistance, observed 15 min following the addition of LTB₄, is highly significant. Corneas were refractory to any further addition of LTB₄ following the initial addition of LTB₄ (10⁻⁷ M). However, when LTB₄-refractory corneas were given LTC₄ (10⁻⁷ M), typical LTC₄ stimulation of the SCC was observed. Likewise, LTC₄-refractory corneas yielded the typical LTB₄ suppression of the SCC, indicating that saturation of the effect of one leukotriene has no effect on the response to another. As was the case with LTC₄, addition of LTB₄ to the endothelial side of the chamber yielded no effect on the SCC or PD. Subsequent addition of LTB₄ to the endothelial side of the same corneas yielded the typical suppression of the SCC and PD, indicating that only endothelial additions were effective.

Effect of FPL 55712 (10⁻⁵ M): FPL 55712, a receptor antagonist of LTC₄, LTD₄, and LTE₄, was added to the corneal chamber. In three separate experiments, the addition of LTC₄ (10⁻⁷ M) 10 min after the antagonist, yielded no change in the SCC or PD as can be seen in Figure 3. Control corneas, without FPL 55712, gave the expected stimulation of SCC and PD with LTC₄. The addition of FPL 55712 alone yielded a small transient downward response with a rapid return to
the original baseline, as shown in Figure 3. The addition of LTB4 resulted in the typical suppression of SCC in the FPL 55712-treated cornea, as well as the control cornea. Thus, FPL 55712 entirely blocked the response to LTC4 but had no effect on the LTB4 response.

**LTD4, LTE4 and 5-HETE**: When other synthetic leukotrienes were tested for their effect on the SCC, it was found that addition of LTD4 or LTE4 to the endothelial side of the chamber, yielded only a relatively small stimulation, even at $10^{-6} \text{M}$ (Fig. 4). The addition of 5-HETE to the chamber yielded a suppression of SCC. The response is reminiscent of that produced by LTB4, but 5-HETE appears to be less potent (Fig. 5).

**Absence of LTC4 effect in chloride-free Ringer's**: In order to determine whether the stimulation caused by LTC4 represents an increase in chloride transport, an experiment was performed with sulfate Ringer's containing no chloride (Fig. 6). When the SCC had stabilized, LTC4 was added to the chamber. No increase in SCC was obtained with LTC4 ($10^{-7} \text{M}$) under these conditions. When sulfate Ringer's was washed out and replaced by chloride-containing Ringer's, and the SCC was allowed to stabilize at a new baseline, LTC4 was again added. This time an immediate stimulation of SCC was observed indicating that the cornea was viable and capable of responding normally to stimulation.

That no stimulation was obtained in chloride-free Ringer's indicates that chloride ions are required for the response, strongly suggesting that LTC4 stimulates active chloride transport.

**Discussion**

The experiments described in this paper show that the isolated frog cornea has the capacity to form metabolites representing both the CO and LO branches of the "AA cascade," when incubated with exogenous AA. Of the metabolites found by TLC, PGE2, and LTB4 predominate. It should be noted that the TLC technique does not permit the isolation of the peptido-leukotrienes (LTC4, LTD4, and LTE4). However, we have confirmed the biosynthetic capacity of the cornea to produce LTC4 by radioimmunoassay.

These experiments also demonstrate that the leukotrienes are highly potent modulators of active chloride transport when added to the Ussing-type chambers at $10^{-7} \text{M}$. Although this concentration range may appear to be high for leukotrienes, the actual concentration that ultimately reaches the basolateral surface of
the corneal epithelium is probably much lower. The data indicate that the corneal epithelial surface is a complete barrier to leukotriene entry. Therefore, for leukotrienes to reach the site of active transport, they must first travel through the entire thickness of the endothelium and stroma. Although our data show that this diffusion occurs within several minutes, the leukotrienes are known to be extremely labile, rapidly oxidizing to inactive degradation products, and it is likely that most of the applied leukotrienes degrade to an inactive form as they diffuse through the endothelium and stroma. Therefore, the effective concentration within the corneal epithelial cells is probably very much lower than the applied dosage.

Our finding that LTB₄ and LTC₄ have virtually equal but opposite effects on SCC and PD is quite remarkable, and the possible biological significance of these leukotrienes as endogenous regulators of Cl⁻ transport is of great interest. Since the corneal epithelium has been shown to be the site of active chloride transport, endogenously produced leukotrienes must travel through both the endothelial and stromal layers of the cornea before arriving at the epithelium where they exert their effect. Our experiments indicate that this can occur rapidly because the lag time from addition of leukotrienes until the onset of the effect is about 3 min. The data showing that eicosanoid synthesis occurs in corneal endothelium are interesting in this regard. Eicosanoids synthesized by corneal endothelium would be able to rapidly diffuse through to the epithelium and, once there, modulate active transport. Any chemical signal capable of stimulating phospholipase A₂ activity would cause the release of free AA from phospholipid, resulting in eicosanoid formation, ultimately modulating active Cl⁻ transport across the cornea.

Our experiments with FPL 55712, the leukotriene receptor antagonist, show that LTC₄ stimulation of SCC in cornea is a specific receptor-mediated event. We showed that receptor blockade with FPL 55712 completely antagonized the corneal response to LTC₄ but not to LTB₄. These results suggest that LTB₄ may act on different receptors from those activated by LTC₄ in cornea.

In this study, exogenous AA was incubated with corneal tissue, and metabolite formation was monitored. In previous studies, exogenous AA was added to corneal chambers and a stimulation of the SCC was observed. At that time, stimulation was believed to be due primarily to PGE₂ formation. In light of the present results, it appears likely that stimulation of the SCC by AA may also be due to rapid leukotriene formation from AA substrate. We have observed other bands present on our TLC-autoradiographs, which remain unidentified. The lipoxigenase pathways have yet to be completely characterized, and it is likely that many metabolites remain to be evaluated. Therefore, the possibility remains that other eicosanoids synthesized by the cornea may also contribute to the modulation of chloride transport.

These experiments demonstrate the existence in the isolated frog cornea of the cellular machinery needed for the biosynthesis of lipoxigenase pathway metabolites. We have also demonstrated that the cornea has the biosynthetic capacity to produce both LTB₄ and LTC₄. Because these leukotrienes have the ability to either depress or elevate active chloride transport in cornea, the possibility exists that they may function as endogenous regulators of this process.

Key words: arachidonic acid, leukotrienes, chloride transport corneal epithelium, prostaglandins

Acknowledgments

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References