Estrogens induce lipoxygenase derivative formation in rabbit lens

Mauricio Guivernau,* Alicia Terragno, Michael W. Dunn, and Norberto A. Terragno

The effect of estrogens on arachidonic acid metabolism was studied in lenses from normal and sex hormone-treated female rabbits. Analysis of the metabolites derived from incubation of these lenses with (1-14C) arachidonic acid provides the first evidence that estradiol activates the lipoxygenase pathway, as indicated by 5-S-hydroxy-6,8,11,14-eicosatetraenoic acid–like product formation, an effect that can be prevented by progestin treatment. Prostaglandin synthesis was not demonstrated by the control or estrogen-treated rabbit lenses, suggesting that lipoxygenase is the major arachidonic acid pathway in the lens. (INVEST OPHTHALMOL VIS SCI 32: 214-217, 1982.)

Key words: lens, arachidonic acid, prostaglandin, lipoxygenase, HETE, estrogens, progestins

The ability of the lens to convert exogenous arachidonic acid (AA) into prostaglandins (PGs) has not been clearly demonstrated despite the abundance of phospholipids and the presence of phospholipase activity in this tissue. Whether AA can be metabolized in the lens via the lipoxygenase pathway has not been determined, although lipoxygenase activity has recently been described in other ocular tissues such as rabbit conjunctiva and iris and calf cornea. The lipoxygenase pathway, first described in platelets by Hamberg and Samuelsson in 1974, is the alternative major pathway for AA metabolism, giving rise to derivatives, including labile hydroperoxides, two stable hydroxy acids, 12-HETE and 5-HETE, and the leukotrienes, which have been proposed as mediators of the anaphylactic response. We have recently reported that estrogens activate AA metabolism via the lipoxygenase pathway in gingival tissue. This investigation has been performed to determine whether nonprostanoic metabolism of AA is present in the lens, with estrogens as stimulating agents of lipoxygenase enzymatic activity.

Materials and methods

The drugs used in this study were obtained from the following sources: (1-14C) AA from New England Nuclear; ethynyl estradiol, norethindrone, indomethacin, 5, 8, 11, 14-eicosatetraenoic acid (ETYA), and nordihydroguaiaretic acid (NDGA) from Sigma Chemical Co.; PGs from the Upjohn Co.; solvents from Fisher Scientific Co.; thin-layer chromatography plates from Macherey-Nagel; and X-ray film from Kodak Co. Hydroxy fatty acid standards were a gift of Dr. Lawrence Levine, Brandeis University, Boston.
Fig. 1. Radiochromatogram scan of the lipid extract of lenses incubated with (1-14C) arachidonic acid from vehicle-treated control rabbits (A), ethynyl estradiol-treated rabbits (B), ethynyl estradiol-treated rabbits in the presence of indomethacin (C), and rabbits treated with ethynyl estradiol plus norethindrone (D). The spots on the bottom represent the migration of the different standards. The phospholipids (PL) remained at the origin. The scans were run at the same sensitivity.

Two synthetic steroids, ethynyl estradiol and norethindrone, were given to New Zealand female rabbits (2 to 3 kg) as an intramuscular depot injection at 0 and 7 days of the study. One experimental group (n = 7) received 140 μg of ethynyl estradiol per week; another group (n = 7) received 140 μg of ethynyl estradiol and 3 mg of norethindrone per week in separate injection sites; the third group (n = 5) was the control and received vehicle only.

Animals were sacrificed by intracardiac pentobarbital injection on day 14 of treatment; the intact lenses were removed immediately and placed in ice-cold Krebs' solution. Lenses were pooled and cut into pieces, and samples of about 1.5 gm were incubated in 7.5 ml of Krebs' solution (pH 7.4) containing 0.5 μCi of AA (specific activity 55 mCi/mmol) in ethanolic solution for 3 hr at 37° C. The reaction was terminated by acidification with 0.375 ml of citric acid (1M). The combined incubation mixture was extracted three times with chloroform and dried under a stream of nitrogen. The extraction residue, redissolved in chloroform:methanol (2:1), was applied to a silica gel G thin-layer chromatographic (TLC) plate (0.25 mm, 20 by 20 cm) on which standards of authentic PG, HETE, and AA were also spotted. The TLC plates were developed twice, as described by Sun et al., in a solvent system consisting of ethyl acetate:2,2,4-trimethylpentane (iso-octane):acetic acid:water (66:30:12:60, v/v). This technique clearly separates all the AA derivatives mentioned above. Standards were visualized by spraying the TLC plates with an ethanolic solution of phosphomolybdic acid (10%) and heating to 37° C for 10
Table I. Effect of sex hormones on the metabolism of (1-^14C) AA in rabbit lens*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Phospholipids</th>
<th>Prostaglandins</th>
<th>5-HETE</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.4 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>98.6 ± 0.7</td>
</tr>
<tr>
<td>Ethynyl estradiol</td>
<td>2.0 ± 0.5</td>
<td>ND</td>
<td>44.8 ± 2.8</td>
<td>53.2 ± 2.0</td>
</tr>
<tr>
<td>Ethynyl estradiol + indomethacin</td>
<td>2.5 ± 0.2</td>
<td>ND</td>
<td>51.5 ± 1.6</td>
<td>46.0 ± 1.3</td>
</tr>
<tr>
<td>Ethynyl estradiol + ETYA</td>
<td>0.5 ± 0.02</td>
<td>ND</td>
<td>ND</td>
<td>99.5 ± 0.01</td>
</tr>
<tr>
<td>Ethynyl estradiol + norethindrone</td>
<td>0.7 ± 0.5</td>
<td>ND</td>
<td>ND</td>
<td>99.3 ± 0.02</td>
</tr>
</tbody>
</table>

ND = not detectable.
*Values represent the mean ± S.E.M. of the percentage of total radioactivity recovered.

Table I. Effect of sex hormones on the metabolism of (1-^14C) AA in rabbit lens*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Phospholipids</th>
<th>Prostaglandins</th>
<th>5-HETE</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.4 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>98.6 ± 0.7</td>
</tr>
<tr>
<td>Ethynyl estradiol</td>
<td>2.0 ± 0.5</td>
<td>ND</td>
<td>44.8 ± 2.8</td>
<td>53.2 ± 2.0</td>
</tr>
<tr>
<td>Ethynyl estradiol + indomethacin</td>
<td>2.5 ± 0.2</td>
<td>ND</td>
<td>51.5 ± 1.6</td>
<td>46.0 ± 1.3</td>
</tr>
<tr>
<td>Ethynyl estradiol + ETYA</td>
<td>0.5 ± 0.02</td>
<td>ND</td>
<td>ND</td>
<td>99.5 ± 0.01</td>
</tr>
<tr>
<td>Ethynyl estradiol + norethindrone</td>
<td>0.7 ± 0.5</td>
<td>ND</td>
<td>ND</td>
<td>99.3 ± 0.02</td>
</tr>
</tbody>
</table>

ND = not detectable.
*Values represent the mean ± S.E.M. of the percentage of total radioactivity recovered.

Results

Control lenses from vehicle-treated rabbits incubated with (1-^14C) AA for 3 hr at 37°C were not able to convert AA into radiolabeled PGs. A radiochromatogram scan was shown in Fig. 1, A. Lens from rabbits pretreated with ethynyl estradiol for 14 days shows a striking difference when compared with the control animals; the presence in the radiochromatogram scan of a clear peak (Fig. 1, B) with similar chromatographic properties to 5-,6-,7-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE), a nonprostaglandin product of the AA metabolism produced by the lipoxygenase pathway. Additional characterization of this 5-HETE-like material was obtained by studying its chromatographic mobility in other solvent systems. The zone corresponding to 5-HETE was scraped, extracted, and rechromatographed in two different solvent systems consisting of chloroform:methanol:acetic acid:water (90:6:1:0.75, v/v) or petroleum ether:ether:acetic acid (50:50:1, v/v), respectively.

To further characterize the HETE peak, lenses from animals treated with ethynyl estradiol were incubated with (1-^14C) AA in the presence of the lipoxygenase inhibitors, NDGA or ETYA, or the cyclooxygenase inhibitor, indomethacin. The formation of this 5-HETE-like product was abolished by NDGA (30 μM) and ETYA (50 μM). However, indomethacin (5.5 μM) did not inhibit it; moreover, the HETE synthesis increased after inhibition of the cyclooxygenase pathway, as can be seen in Table I and Fig. 1, C. This evidence provided further support that the product formed from AA under these experimental conditions is a lipoxygenase derivative.

The radioconversion of (1-^14C) AA by lens from rabbits treated with ethynyl estradiol plus norethindrone is seen in Fig. 1, D. This radiochromatogram scan shows that the large peak observed in animals treated with estradiol alone and characterized as 5-HETE is not present here. Thus addition of progestins to the estrogen treatment prevents the HETE formation. Although the mechanism of the progestin effect remains to be elucidated, we have also observed a similar interaction in the estrogen-induced alteration on platelet reactivity affecting the cyclooxygenase pathway.**

Discussion

The role of estrogens in AA turnover affecting the cyclooxygenase pathway has been observed in several tissues. Our results provide the first evidence that estrogens induce activation of the lipoxygenase pathway in the lens, as demonstrated by HETE-like product formation. Addition of progestins to estrogens has been shown to inhibit the formation of HETE.
gen-treated rabbits prevents activation of this pathway and HETE synthesis. This finding agrees with our recent report that estrogens are able to induce synthesis of lipoxygenase products in gingival tissue. Furthermore, the activation of a quiescent nonprostanoic pathway of the AA metabolism in the lens raises the interesting possibility that other stimuli can induce the same effect and that lipoxygenase derivatives can have important effects on lens function, as has been demonstrated in platelets and mast cells. Although the biologic role of the lipoxygenase products have as yet been only partially elucidated, they have been reported to be potent chemotactic agents for human polymorphonuclear leukocytes, substances able to enhance anaphylactic mediator release, alter membrane components, and increase vascular permeability. These known actions strongly suggest a possible role of hydroxy fatty acids in the mediation of lens induced intraocular inflammation. The last possibility is especially appealing considering both the lack of blood vessels and the inability of the lens to synthesize other inflammatory mediators such as prostaglandins. Further investigation of the pathophysiologic role of this lipoxygenase derivative is needed before its clinical significance can be established.

We thank Miss Lillian Delgado and Miss Jeannie Maura for their help in preparing this manuscript. We also thank Dr. Lawrence Levine, Brandeis University, for donating the 5-HETE standard and Mr. Michael R. LaPorta from Merck, Sharp & Dohme for library search services.

REFERENCES

17. Stenson WF and Parker CW: 12-l-Hydroxy-5,8,10,14-eicosatetraenoic acid, a chemotactic fatty acid, is incorporated into neutrophil phospholipids and triglyceride. Prostaglandins 18:283, 1979.