Rat lens microsomal preparations possess the capability of converting exogenous arachidonic acid into prostaglandins (PG) E2 and F2α. The low, yet measurable prostaglandin biosynthetic capacity of the lens microsomes was demonstrated by radioimmunoassay (RIA) and by separation of radiolabeled products after incubation with high specific activity U-[14C]-arachidonic acid. Maximal formation of radioimmunoassayable PGE2 and PGF2α was measured after a 15-min incubation at 37°C with approximately 0.5 mg protein. Prostaglandin biosynthesis was inhibited by the nonsteroidal anti-inflammatory agents aspirin (IC50 = 52 nM) and indomethacin (IC50 = 20 μM). These results unequivocally demonstrate prostaglandin biosynthesis in rat lens.

Investigations into whether the mammalian lens has the capacity to synthesize prostaglandins (PGs) have been inconclusive. Van Dorp et al1 and Belisle et al2 have presented evidence suggesting a low, yet measurable lens PG biosynthetic capacity. However, other investigations have raised serious doubt concerning the ability of the lens to generate PGs. Kass and Holmberg3 and Guivernau et al4 found no PG production by rabbit lens microsomes using radiocchemical techniques. Taylor et al5 were unable to detect PGs in the medium of cultured calf lens epithelial cells by radioimmunoassay (RIA). Since no general agreement had been reached concerning the capability of the lens to synthesize these important bioregulatory molecules, further studies of this issue were initiated. Recent refinements in the methods used to detect and measure minute amounts of PGs6,7 were employed in this study. We report here that lens microsomal preparations from young Sprague-Dawley rats do synthesize PGE2 and PGF2α from exogenous arachidonic acid.

Materials and Methods

Universally labeled U-[14C]-arachidonic acid (360 mCi/mmol), [3H]-PGF2α (160 Ci/mmol), and [3H]-PGE2 (150 Ci/mmol) were purchased from New England Nuclear (Boston, MA). PGE2, PGF2α, and PGD2 were obtained from Upjohn Diagnostics (Kalamazoo, MI). Unlabeled arachidonic acid was purchased from Nu-Chek-Prep (Elysian, MN). Bovine serum albumin (fatty acid poor) was obtained from Calbiochem (La Jolla, CA). Indomethacin was kindly supplied by Dr. Philip Davies of Merck, Sharp and Dohme (Rahway, NJ). Aspirin and reduced glutathione were purchased from Sigma Chemical (St. Louis, MO).

Animals were housed and utilized in accordance with the ARVO resolution on the Use of Animals in Research. Data is expressed as the mean ± the standard error of the means (SEM). Regression analysis was used for determination of linearity.

Protein was determined by the method of Lowry et al8 using bovine serum albumin as standard.

Microsomal Preparation from Rat Lens

The microsomal fraction from enucleated lenses of young (21- to 42-day-old) Sprague-Dawley rats were prepared as previously described.7 The lenses, 12-16, were homogenized in three volumes of cold 0.1 M potassium phosphate buffer (KPO4), pH 7.4, containing 1% fatty acid poor bovine serum albumin (BSA) and 10 mM EDTA. The homogenate was centrifuged at 8,000 × g for 15 min and the resulting supernatant centrifuged at 100,000 × g for 60 min. The pellets were washed extensively with 0.1 M KPO4 buffer, pH 7.4, and suspended in the same buffer. The microsomal preparations were used within 30 min. BSA and EDTA were included in the homogenizing medium to protect the labile cyclooxygenase enzyme from autocatalytic destruction.9 BSA scavenges fatty acids which may be released during tissue handling while EDTA may limit phospholipase activity by chelating calcium.10
Figure 1. Autoradiography of a thin layer chromatographic plate demonstrating rat lens prostaglandin production from \( \text{U-[^{14}C]} \)-arachidonic acid. Microsomes (MIC) were incubated with 1 mM reduced glutathione (GSH), 1 mM epinephrine (EPI), and 100,000 cpm \( \text{U-[^{14}C]} \)-arachidonic acid (10 \( \mu \text{M} \)) in a total volume of 30 \( \mu \text{L} \) (lane 1). Boiling the microsomal preparation prior to incubation with labeled arachidonic acid eliminated PG formation (lane 2). A reaction mixture, without microsomes, was incubated to serve as an arachidonic acid (AA) blank (lane 3).

**Incubation With \( \text{U-[^{14}C]} \)-Arachidonic Acid and Isolation of Radiolabeled Prostaglandins**

Microsomes (115 \( \pm \) 37 \( \mu \text{g} \) protein) were incubated with 1 mM reduced glutathione, 1 mM epinephrine and \( \text{U-[^{14}C]} \)-arachidonic acid (10 \( \mu \text{M} \), 100,000 cpm) in a total volume of 30 \( \mu \text{L} \) for 15 min at 37°C. The reaction was terminated with 20 \( \mu \text{L} \) 4N formic acid and extracted three times with 100 \( \mu \text{L} \) ethyl acetate. The organic solvent was evaporated under nitrogen and the residue redissolved in chloroform:methanol (2:1 v/v). The sample was applied to silica Gel G plates and developed in System C of Nugteran and Hazelhof (chloroform:methanol:water:acetic acid; 100:10:1:0.8 v/v). Authentic PGs were co-chromatographed with the samples and visualized after plate development with iodine vapor. After autoradiography (10-day exposure), the plates were cut and the bands corresponding to the PG standards and arachidonic acid were counted. Background levels of radioactivity associated with the development of the plate were subtracted from experimental values to obtain measurements of PG biosynthesis. Extraction efficiency was between 65 and 75%.

**Determination of Prostaglandin Biosynthesis by Radioimmunoassay**

The microsomal preparations (458 \( \pm \) 47 \( \mu \text{g} \) protein) were incubated with 1 mM reduced glutathione, 1 mM epinephrine and 200 \( \mu \text{M} \) arachidonic acid at 37°C for 15 min. Reactions were terminated by the addition of 50 \( \mu \text{L} \) 4N formic acid and the acidified medium was extracted three times with 400 \( \mu \text{L} \) ethyl acetate. The organic solvent was evaporated under a stream of nitrogen and the residue was resuspended in RIA buffer (0.9% NaCl, 0.1% BSA, 50 mM KPO4 buffer, pH 7.4). RIAs were performed by the method of Reingold et al.\(^{12}\) using antisera generated in rabbits.\(^{13}\) Arachidonic acid was found not to interfere with the RIA at the concentrations employed in this study. The 50% binding for \( \text{PGE}_2 \) and \( \text{PGF}_{2\alpha} \) were 48 pg and 17 pg, respectively. The lower limits of detection, defined as a 10% shift in labeled ligand binding, were 10 pg for \( \text{PGE}_2 \) and 2 pg for \( \text{PGF}_{2\alpha} \), respectively. Blanks, consisting of all incubation components except either arachidonic acid or microsomes, were run in each experiment and background values were routinely subtracted from the experimental values. To demonstrate enzymatic synthesis of PGs in the preparations, microsomes were preincubated with the cyclooxygenase inhibitors aspirin or indomethacin for 5 min at 22°C. Indomethacin was dissolved in 0.1 M Tris-acetate buffer, pH 8.0. Aspirin was prepared in a 20% ethanol solution. The final concentration of ethanol in the incubation mix was less than 0.3%. Ethanol alone did not affect PG biosynthesis or interfere with RIA.

**Results**

 Autoradiography of thin layer chromatographic plates revealed that the rat lens microsomes, when incubated with high specific activity \( \text{U-[^{14}C]} \)-arachidonic acid, synthesized radiolabeled products which co-migrated with authentic \( \text{PGE}_2 \), \( \text{PGF}_{2\alpha} \) and \( \text{PGD}_2 \) (Fig. 1). Product formation was completely eliminated by boiling the microsomal preparations prior to incubation with labeled substrate, which demonstrated that the products were enzymatically generated. Under the conditions of assay employed, approximately 1.5% \((n = 3)\) of the labeled substrate was converted into products by the lens microsomal preparations. These initial experiments were conducted with low amounts of microsomal protein (115 \( \mu \text{g} \) sample) and a 15-min incubation period to obtain a profile of the PGs synthesized by the lens microsomes.
Subsequent studies utilized the radioimmunoassay to verify and quantitate PG formation. RIA measurements revealed that PG biosynthesis was directly proportional to the amount of lens microsomal protein employed in an incubation (Fig. 2). Preincubation of the microsomal preparation with 1 mM aspirin completely inhibited PG formation in these experiments. PGF₂α biosynthesis exceeded PGE₂ production even in the presence of reduced glutathione, a required cofactor for the PGH₂ → PGE₂ isomerase. The rate of product formation was 49 ± 4 pg PGE₂/mg protein/min and 151 ± 20 pg PGF₂α/mg protein/min (n = 4–6). Calculated on a per lens basis, 102 ± 13 pg PGE₂ and 158 ± 29 pg PGF₂α were produced during the 15-min incubation of the lens microsomes with 200 μM arachidonic acid. The higher levels of PGF₂α could have been the result of PGE₂-9-ketoreductase activity which would convert PGE₂ into PGF₂α. However, no PGF₂α production was observed after incubation of the lens microsomes with 1 μg [³H]-PGE₂ and 3.3 mM reduced NADPH. These results are consistent with the finding that the PGE₂-9-ketoreductase is a cytosolic enzyme and probably not present in the lens microsomal preparations.

When microsomal preparations were incubated with varying concentrations of arachidonic acid, PGF₂α biosynthesis was found to be inhibited by high concentrations of substrate (Fig. 3). PGF₂α biosynthesis rose sharply and reached a maximum at approximately 50 μM arachidonate. At substrate levels above 50 μM, PGF₂α production declined to 65% of maximum. In contrast, PGE₂ biosynthesis continued to rise as the substrate concentration was increased to 200 μM. Differences in the biosynthesis of PGE₂ and PGF₂α have been reported in lung and sheep seminal vesicles. These results suggest that a substrate concentration of between 20 and 50 μM would prove most useful in studying lens arachidonate metabolism.

Prostaglandin biosynthesis was inhibited in a dose-dependent manner by the cyclooxygenase inhibitors aspirin and indomethacin (Fig. 4). The concentration of indomethacin required to produce a 50% inhibition (IC₅₀) of PGF₂α biosynthesis was 20 μM. Indomethacin was a more effective inhibitor of lens PG biosynthesis than aspirin which had an IC₅₀ of 52 μM. The cyclooxygenase inhibitors were also effective in inhibiting PGE₂ biosynthesis in the lens microsomal preparations. The IC₅₀ for PGE₂ was 25 μM indomethacin and 48 μM aspirin.

Discussion

Our results demonstrate that microsomes prepared from rat lenses possess the capacity to generate PGE₂ and PGF₂α from exogenous arachidonic acid. Product formation was quantified by RIA and confirmed by autoradiography. Specific inhibitors of the cyclooxygenase enzyme effectively limited the generation of the arachidonic acid metabolites in the lens microsomal preparations.

The low capacity of the rat lens to produce PGs and the limited sensitivity of the procedures previously used to assay for lens PG synthesis, are the probable reasons for the discrepancy observed in the literature.

Fig. 2. Relationship between lens microsomal protein concentration and prostaglandin production. Microsomes from eight to 12 lenses were prepared and diluted with 0.1 M potassium phosphate buffer. Undiluted aliquots of the microsomal preparations were preincubated with 1 mM aspirin (ASA). PGE₂ (•) and PGF₂α (•) levels were determined by RIA. Individual data points from three separate experiments are plotted.

Fig. 3. Prostaglandin production in rat lens microsomes incubated with different concentrations of arachidonic acid. Arachidonate concentrations ranging from 0 to 200 μM were used in a 15-min incubation period. PGE₂ (•) and PGF₂α (•) production were determined by RIA and expressed as a percent of the maximum product formation. Maximum production was 604 ± 178 pg PGF₂α/mg protein and 333 ± 82 pg PGE₂/mg protein at 50 and 200 μM fatty acid, respectively. Values are the mean ± SEM of three determinations.
regarding the ability of the lens to generate PGs. With the introduction of an assay which utilized high specific activity U-\[\text{14C}\]-arachidonic acid and thin layer chromatographic separation of labeled products, PG biosynthesis is demonstrated, further substantiating the studies performed with RIA. While low cyclooxygenase activity may be a principal reason for the limited lens PG production, age and species differences may also be determining factors.

The synthesis of PGD2 by the lens microsomes (shown in Fig. 1) was unexpected and awaits confirmation. While the enzymatic conversion of arachidonic acid to PGD2 normally requires PGH2 \(\rightarrow\) PGD2 isomerase, a cytosolic enzyme, it is possible that the PGH2 \(\rightarrow\) PGD2 isomerase may be associated with the microsomal fraction obtained from the lens. However, the more likely possibility is that the PGD2 was formed spontaneously from the nonenzymatic degradation of PGH2 as described by Christ-Hazelhof and Nugteran. The possible production of PGD2 by the lens is intriguing in light of the report suggesting the limited lens PG production, age and species differences may also be determining factors.

The demonstration of PG biosynthesis by rat lens microsomal preparations, together with the recent report of leukotriene B4 production in bovine lens, suggests that the arachidonic acid cascade is present in lens tissue and may function to regulate certain biochemical events.

**Fig. 4.** The effect of cyclooxygenase inhibitors on rat lens microsomal PGF2a biosynthesis. Aspirin (ASA; □) and indomethacin (INDO; ○) were preincubated with rat lens microsomes as described in the Methods section. Maximum production of PGF2a was 465 ± 165 pg/mg protein in the aspirin pretreated experiments and 592 ± 142 pg/mg protein in the indomethacin study. Values are the means ± SEM of three to five determinations.

**Key words:** rat lens, prostaglandins, aspirin, indomethacin

**References**