Biosynthesis of Prostaglandins from Arachidonic Acid in Guinea Pig Lung

PROSTAGLANDINS AND RELATED FACTORS 38*

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The prostaglandins constitute a class of physiologically active compounds with a widespread occurrence in animal tissues (see References 2 and 3). Previous studies have led to the determination of their structures (4-6), and more recent investigations have shown that they can be formed enzymatically from the essential fatty acids (7, 8). It was thus shown that in homogenates of vesicular glands from sheep, prostaglandin E₁ (PGE₁)1 is formed from 8, 11, 14-eicosatetraenoic acid (9, 10), PGE₂ from 5, 8, 11, 14-eicosatetraenoic acid (7, 8), and PGE₃ from 5, 8, 11, 14, 17-eicosapentaenoic acid (10). However, no PGF-compounds were detected.

The experiments reported here, show that the biosynthesis of prostaglandins is not restricted to the vesicular gland, but it can also take place in homogenates of guinea pig lung. In this system, both PGF₂α and PGE₂ are formed from arachidonic acid.

EXPERIMENTAL PROCEDURE

Chromatographic Methods—Reversed phase partition chromatography was performed as described earlier (11). The solvent systems used are given in Table I.

Thin layer chromatography on AgNO₃-impregnated silica gel was performed as previously described (12). The solvent systems are given in Table II. The substances were detected by spraying with concentrated sulfuric acid and heating to 150–200°.

Gas-liquid chromatography was performed with an F and M Biomedical gas chromatograph model 400 equipped with a hydrogen flame ionization detector and a stream splitter. A gas chromatography fraction collector (Packard), with cartridges containing crystals of p-terphenyl, was used to trap the effluent for determination of radioactivity (13).

Lung Preparation—Lungs from adult male guinea pigs were rapidly excised and minced in ice-cooled medium consisting of a modified Bucher medium (20 mM KH₂PO₄, 72 mM K₂HPO₄, and 27.6 mM nicotinamide, and 3.6 mM MgCl₂, pH 7.4). Homogenates with a tissue to buffer ratio of 1:4 were prepared in a Potter-Elvehjem type homogenizer with a loosely fitting Teflon pestle. The homogenate was centrifuged at 900 × g for 15 min and the supernatant fluid was used. The tritium-labeled arachidonic acid was dissolved in benzene and added to the incubation flasks. After the solvent had been evaporated by a stream of nitrogen, the lung homogenate was added to give a concentration of arachidonic acid of about 6 μg per ml. The incubation was carried out aerobically at 37° with shaking. After 30 min, the incubation was terminated by addition of 9 volumes of 96% aqueous ethanol. The solution was filtered, evaporated to a small volume under reduced pressure, acidified to pH 3 with 2 N HCl, and extracted three times with ether. The combined ether extracts were washed with water until neutral and evaporated to dryness. The residue was chromatographed together with 5 mg of cholic acid or 0.5 mg of PGE₂ on a 4.5-g column of hydrophobic Hyflo Super-Cel with Solvent C-47. The cholic acid, which in this system appears 5 to 10 ml after PGE₂ was detected by titration with 0.02 N NaOH. The PGE₂ was detected by measuring the ultraviolet absorption at 278 nm after treating aliquots of fractions with 0.5 N aqueous sodium hydroxide.

Material—Tritium-labeled arachidonic acid (95% purity) with a specific activity of 9 μCi per mg (7) was a gift from Dr. D. A. van Dorp, Unilever Research Laboratories, Vlaardingen, Holland.

RESULTS

When tritium-labeled arachidonic acid was incubated with a homogenate of guinea pig lung, about 10% of the radioactivity was converted into products with chromatographic properties resembling prostaglandins. These products were not observed in a control experiment with boiled homogenate. A chromatogram of an extract from one of the incubations is shown in Fig. 1. The main peak (I) of radioactivity had an elution volume characteristic of PGF₂α. Three small peaks (II, III, and IV) appeared later in the chromatogram.

Identification of PGF₂α—The radioactive material present in the main peak (I in Fig. 1; 43 to 62 ml of effluent) was further analyzed by thin layer chromatography on AgNO₃-impregnated silica gel with Solvent A III. Practically all of the radioactivity was found within the area of the reference sample of PGF₂α. Treatment of part of the isolated material with diazomethane gave a radioactive product, which on a AgNO₃-impregnated

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**Table I**

Solvent systems used in reversed phase partition chromatography*

<table>
<thead>
<tr>
<th>System</th>
<th>Moving phase</th>
<th>Stationary phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-47</td>
<td>Methanol-water (140:160)</td>
<td>Chloroform-isooctanol (15:15)</td>
</tr>
<tr>
<td>F-53</td>
<td>Methanol-water (159:141)</td>
<td>Chloroform-heptane (45:15)</td>
</tr>
</tbody>
</table>

* The composition of the solvent systems is given in milliliters.

**Table II**

Solvent systems used in thin layer chromatography*

<table>
<thead>
<tr>
<th>System</th>
<th>Composition</th>
<th>Adsorbent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A I</td>
<td>Benzene-dioxane-acetic acid (40:40:2)</td>
<td>Silica Gel G†</td>
</tr>
<tr>
<td>A III</td>
<td>Ethyl acetate-acetic acid-2,2,4-trimethylpentane-water (110:10:30:100)‡</td>
<td>Silica Gel G-AgNO₃ (25:1)</td>
</tr>
<tr>
<td>M II</td>
<td>Ethyl acetate-methanol-water (80:20:50)§</td>
<td>Silica Gel G-AgNO₃ (30:1)</td>
</tr>
</tbody>
</table>

* The composition of the solvent systems is given in milliliters.
† Merck AG, Germany.
‡ The solvent mixtures were equilibrated for 1 hour and the upper phase was used.
§ The solvent mixtures were equilibrated for 1 hour and the upper phase was used.

The results showed that the labeled material was identical with PGF₃₀. The yield of PGF₃₀ from arachidonic acid was calculated to be about 5%. (No correction was made for loss of tritium in the conversion to PGF₃₀.)

**Identification of PGE₂**—The radioactive material appearing just before the cholic acid in the chromatogram shown in Fig. 1

![Figure 1](http://www.jbc.org/)

**Fig. 1.** Reversed phase partition chromatography of an extract (see under "Experimental Procedure") from an incubation of 180 µg of labeled arachidonic acid with 30 ml of a homogenate of guinea pig lung. Conditions: column, 4.5 g of hydrophobic Hyflo Super-Cel; solvent, System C-47.

silica gel (Solvent M II) had the same mobility as the methyl ester of PGF₃₀.

On thin layer chromatography on silica gel without AgNO₃ (Solvent A I), the radioactive material was again indistinguishable from PGF₃₀. Further evidence as to the identity of the labeled material was obtained by gas-liquid chromatography. Unlabeled PGF₃₀ (10 µg) was added to one-third of the radioactive material from the chromatogram seen in Fig. 1. The product was treated with diazomethane, and it was converted into trimethylsilyl ether derivatives (14). A gas-liquid chromatogram with simultaneous recording of mass and radioactivity of this material is shown in Fig. 2. The peak due to the added PGF₃₀ coincided with the main peak of radioactivity.

![Figure 2](http://www.jbc.org/)

**Fig. 2.** Gas-liquid chromatography of PGF₃₀ and radioactive material in Peak I (43 to 62 ml of effluent) of the chromatogram shown in Fig. 1. The material was treated with diazomethane and converted to trimethylsilyl ether derivatives (14). Conditions: column, glass tube with 0.75% SE-30 on 100 to 120 mesh Gas Chrom P, 3 mm × 1.6 m; flash heater temperature, 230°; column temperature, 185°; carrier gas pressure, 3.0 Kg per cm². The effluent gas was divided by a stream splitter. One-tenth of the effluent reached the hydrogen flame ionization detector. The remaining nine-tenths of the effluent gas were diverted to a fraction collector for determination of radioactivity (13).

**Fig. 3.** Thin layer chromatography of the radioactive material (II, 84 to 102 ml of effluent; Fig. 1) with unlabeled PGE₂ as reference. Conditions: absorbent, Silica Gel G-AgNO₃ (30:1); solvent, System A III.
had a retention volume characteristic of PGE$_2$. This material (II, 84 to 102 ml of effluent) was subjected to thin layer chromatography on AgNO$_3$-impregnated silica gel (Solvent A III) together with unlabeled PGE$_2$. After development, zones of the adsorbent were scraped off, eluted with methanol, and the radioactivity was determined in a liquid scintillation spectrometer. The chromatogram (Fig. 3) showed that the radioactive material had the same mobility as PGE$_2$.

In another experiment, PGE$_2$ was added as a reference in the reversed phase partition chromatography of the incubation extract. A peak of radioactivity coincided with that of the reference sample of PGE$_2$. The radioactive PGE$_2$ fractions were treated with 0.5 N sodium hydroxide at room temperature for 30 min. Under these conditions, PGE compounds are converted to products containing the $\Delta^{12,13}$-unsaturated 9-keto chromophore by elimination of the 11-hydroxyl group and isomerization of the resulting double bond (4, 5). The reaction mixture was acidified with hydrochloric acid and extracted twice with ether. The combined extracts were washed with water until neutral and evaporated in a vacuum. This material was separated by reversed phase partition chromatography with Solvent F-53. A peak of radioactivity coincided with that of the ultraviolet absorption.

The results show that the radioactive material was identical with PGE$_2$.

**Evidence for Formation of Metabolites of PGE$_2$**—The two minor peaks (III and IV), appearing after the cholic acid in the chromatogram shown in Fig. 1, had retention volumes characteristic of 11a,15-dihydroxy-9-ketoprost-5-enoic acid (III in Fig. 4) and of 11a-hydroxy-9,15-diketoprost-5 enoic acid (IV in Fig. 4). These compounds have been shown to be formed from PGE$_2$ by enzymes present in a homogenate of guinea pig lung (1).

When the material present in the two peaks was chromatographed on AgNO$_3$-impregnated silica gel, (Solvent A III) most of the radioactivity was found in zones corresponding to 11a,15-dihydroxy-9-ketoprost-5-enoic acid and 11a-hydroxy-9,15-diketoprost-5 enoic acid, respectively. It appears that under our experimental conditions these compounds were formed from the PGE$_2$ biosynthesized from arachidonic acid (Fig. 4).

**Metabolism of PGF$_{2\alpha}$**—Tritium-labeled PGF$_{2\alpha}$ was prepared by selective reduction of the $\Delta^{12}$-double bond of PGF$_{2\alpha}$ with tritium gas according to a previously described procedure (1) (specific activity, 140 $\mu$C per mg). This material was incubated with a whole homogenate of guinea pig lung in a concentration of 1 $\mu$g per ml. After 30 min at 37°, the labeled product was isolated.

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*K. Grön and B. Samuelsson, unpublished experiments.*
and separated by reversed phase partition chromatography (Solvent C-47) as described above with cholic acid as reference. About 70% of the labeled PGF₂α had been transformed into a less polar compound appearing after cholic acid. The labeled metabolite was further characterized by thin layer chromatography (Solvent A III) and it was shown to be different from PGE₂ and its metabolites, III and IV (see Fig. 4).

**DISCUSSION**

It is shown in this investigation, that arachidonic acid is converted into prostaglandins in homogenates of guinea pig lung. The products were identified as PGF₂α (I), PGE₂ (II), 11α,15-dihydroxy-9-ketoprost-5-enoic acid (III), and 11α-hydroxy-9,15-diketoprost-5-enoic acid (IV) (see Fig. 4). Previous studies with homogenates of the vesicular gland of sheep only gave evidence for the formation of PGE₂ from arachidonic acid (8).

The formation of III and IV is due to the action of enzymes in the lung homogenate on PGE₂ as recently demonstrated (1). The transformations involve reduction of the Δ₁⁵-double bond, and in the case of IV, oxidation of the secondary alcohol group at C-15 was also involved.

PGF₂α was the main product from arachidonic acid in the lung homogenate. Previous experiments demonstrated that labeled PGE₂ added to the same system is not converted into PGF₂α but only into III and IV (1). Similar experiments showed that PGF₂α is slowly metabolized to material which is not identical with PGE₂, III or IV. These studies indicate that the biosynthesis of PGE₂ and PGF₂α follows pathways, which at least to some extent, are different.

**SUMMARY**

Homogenates of guinea pig lung transformed tritium-labeled arachidonic acid into prostaglandin F₂α, prostaglandin E₂, 11α,15-dihydroxy-9-ketoprost-5-enoic acid, and 11α-hydroxy-9,15-diketoprost-5-enoic acid.

**REFERENCES**

Biosynthesis of Prostaglandins from Arachidonic Acid in Guinea Pig Lung:
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