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 $E_1$ (PGE$_1$) is formed from 8,11,14-eicosatrienoic acid (9, 10), PGE$_2$ from 5,8,11,14-eicosatetraenoic acid (7, 8), and PGE$_3$ from 5,8,11,14,17-eicosapentaenoic acid (10). However, no PGE-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 PGF$_{2\alpha}$ and PGE$_2$ 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 AgNO$_3$-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 $150-200^\circ$.

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 for determination of radioactivity (13), 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 KH$_2$PO$_4$, 72 mM K$_2$HPO$_4$, 27.6 mM sucrose, 0.3 mM Na$_2$HPO$_4$, 1 mM CaCl$_2$, 0.25 mM MgCl$_2$, 10 mM glucose, 10 mM Hepes, 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 9000 x 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 $\mu$g per ml. The incubation was carried out aerobically at 37$^\circ$ with shaking. After 30 min, the incubation was terminated by the addition of 0 volumes of 0.9% 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 PGE$_2$ 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 PGE$_2$, was detected by titration with 0.02 N NaOH. The PGE$_2$ was detected by measuring the ultraviolet absorption at 275 $\mu$m 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 $\mu$C 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 ($I$) radioactivity had an elution volume characteristic of PGF$_{2\alpha}$. Three small peaks ($II$, $III$, and $IV$) appeared later in the chromatogram.

Identification of PGF$_{2\alpha}$—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 AgNO$_3$-impregnated silica gel with Solvent A III. Practically all of the radioactivity was found within the area of the reference sample of PGF$_{2\alpha}$. Treatment of part of the isolated material with diazomethane gave a radioactive product, which on a AgNO$_3$-impregnated
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-iso-octanol (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 results showed that the labeled material was identical with PGF₂α. The yield of PGF₂α from arachidonic acid was calculated to be about 5%. (No correction was made for loss of tritium in the conversion to PGF₂α.)

Identification of PGE₃—The radioactive material appearing just before the cholic acid in the chromatogram shown in Fig. 1...

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. The silica gel (Solvent M II) had the same mobility as the methyl ester of PGF₂α.

On thin layer chromatography on silica gel without AgNO₃ (Solvent A I), the radioactive material was again indistinguishable from PGF₂α.

Further evidence as to the identity of the labeled material was obtained by gas-liquid chromatography. Unlabeled PGF₂α (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 PGF₂α coincided with the main peak of radioactivity.

Fig. 2. Gas-liquid chromatography of PGF₂α 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 0.76% 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 PGE₂. This material (II, 84 to 102 ml of effluent) was subjected to thin layer chromatography on AgNO₃-impregnated silica gel (Solvent A III) together with unlabeled PGE₂. 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 PGE₂.

In another experiment, PGE₂ 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 PGE₂. The radioactive PGE₂ 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 Δ¹²(13,12)-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 PGE₂.

**Evidence for Formation of Metabolites of PGE₂**—The two minor peaks (III and IV), appearing after the cholic acid in the chromatogram shown in Fig. 1, had retention volumes characteristic of 11α,15-dihydroxy-9-ketopro-5-enoic acid (III in Fig. 4) and of 11α-hydroxy-9,15-diketopro-5 enoic acid (IV in Fig. 4). These compounds have been shown to be formed from PGE₂ by enzymes present in a homogenate of guinea pig lung (1).

When the material present in the two peaks was chromatographed on AgNO₃-impregnated silica gel, (Solvent A III) most of the radioactivity was found in zones corresponding to 11α,15-dihydroxy-9-ketopro-5-enoic acid and 11α-hydroxy-9,15-diketopro-5 enoic acid, respectively. It appears that under our experimental conditions these compounds were formed from the PGE₂ biosynthesized from arachidonic acid (Fig. 4).

**Metabolism of PGF₂α**—Tritium-labeled PGF₂α₉ was prepared by selective reduction of the Δ¹²-double bond of PGF₂α with tritium gas according to a previously described procedure (1) (specific activity, 140 μC per mg). This material was incubated with a whole homogenate of guinea pig lung in a concentration of 1 μg per ml. After 30 min at 37°, the labeled product was isolated.

* 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 PGF_{2\alpha} 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 PGE_{2\alpha} 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 PGF_{2\alpha} (I), PGE_{2\alpha} (II), 11\alpha,15-dihydroxy-9-ketoprost-5-enoic acid (III), and 11\alpha-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 PGE_{2\alpha} from arachidonic acid (8).

The formation of III and IV is due to the action of enzymes in the lung homogenate on PGE_{2\alpha} as recently demonstrated (1). The transformations involve reduction of the \Delta^{13}-double bond, and in the case of IV, oxidation of the secondary alcohol group at C-15 was also involved.

PGF_{2\alpha} was the main product from arachidonic acid in the lung homogenate. Previous experiments demonstrated that labeled PGE_{2\alpha} added to the same system is not converted into PGF_{2\alpha} but only into III and IV (1). Similar experiments showed that PGF_{2\alpha} is slowly metabolized to material which is not identical with PGE_{2\alpha}, III or IV. These studies indicate that the biosynthesis of PGE_{2\alpha} and PGF_{2\alpha} follows pathways, which at least to some extent, are different.

**SUMMARY**

Homogenates of guinea pig lung transformed tritium-labeled arachidonic acid into prostaglandin F_{2\alpha}, prostaglandin E_{2\alpha}, 11\alpha,15-dihydroxy-9-ketoprost-5-enoic acid, and 11\alpha-hydroxy-9,15-diketoprost-5-enoic acid.

**REFERENCES**

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