PROSTAGLANDIN ENDOPEROXIDES VII. NOVEL TRANSFORMATIONS OF ARACHIDONIC ACID IN GUINEA PIG LUNG

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SUMMARY: The following labeled compounds were isolated and identified after incubation of [1-^{14}C]arachidonic acid with guinea pig lung homogenates: 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), the hemiacetal derivative of 8-(1-hydroxy-3-oxopropyl)-9,12-dihydroxy-5,10-heptadecadienoic acid (PHD), 12-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), PGE_2, $PGF_{2\alpha}$, 11-hydroxy-5,8,12,14-eicosatetraenoic acid, and 15-hydroxy-5,8,11,13-eicosatetraenoic acid (in order of decreasing yield). Perfused guinea pig lungs released PHD (654-2304 ng), HHT (192-387 ng), HETE (66-111 ng), PGE_2 (15-93 ng), and $PGF_{2\alpha}$ (93-171 ng) following injection of 30 µg of arachidonic acid. Thus guinea pig lung homogenates as well as intact guinea pig lung converted added arachidonic acid predominantly into PHD and HHT, metabolites of the prostaglandin endoperoxide PGG_2, and to a lesser extent into the classical prostaglandins PGE_2 and $PGF_{2\alpha}$.

In a recent study it was found that human blood platelets converted added arachidonic acid into three major products, i.e. 12L-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), 12L-hydroxy-5,8,10-heptadecatrienoic acid (HHT), and the hemiacetal derivative of 8-(1-hydroxy-3-oxopropyl)-9,12L-dihydroxy-5,10-heptadecadienoic acid (PHD)(1). The first mentioned compound was formed from 12L-hydroperoxy-5,8,10,14-eicosatetraenoic acid which was formed from arachidonic acid by a novel fatty acid dioxygenase. The two latter compounds were metabolites of the prostaglandin endoperoxide, PGG_2, which was formed from arachidonic acid by the fatty acid cyclooxygenase involved in prostaglandin biosynthesis. Thrombin-induced aggregation of washed platelets was accompanied by release of large amounts of the three products formed from arachidonic acid. In contrast, the amounts of the classical prostaglandins, PGE_2 and $PGF_{2\alpha}$, were much smaller (2). The present study shows that guinea pig lung produces substantial amounts of HETE, HHT, and PHD from arachidonic acid.

EXPERIMENTAL AND RESULTS

Guinea Pig Lung Homogenate. Guinea pig lungs (2-4 g) were minced in ice-cold 0.1 M potassium phosphate buffer, pH 7.4, (1:3, w/v) containing 4 µg/ml of [1-^{14}C]arachidonic acid and homogenized with a Potter-Elvehjem homogenizer for 2 min. The homogenate was incubated at 37° and aliquots were removed after different times and added to 5 volumes of ethanol. Precipitated protein was filtered off and the solution extracted twice with diethyl ether. The residue containing about 80% of the added radioactivity was treated with diazomethane and subjected to thin layer radiochromatography. Four main peaks of radioactivity appeared, i.e. methyl arachidonate (41% of the recovered radioactivity) and the methyl esters of Compounds I, II, and III (6, 21, and 24%, respectively, of the recovered radioactivity) (Fig. 1). In order to obtain material sufficient for structural work a lung (3 g) was incubated for 5 min with 500 µg of arachidonic acid. The products were isolated as their methyl esters by preparative thin layer chromatography and identified as described below.

The methyl ester of Compound I cochromatographed with methyl 12L-hydroxy-5,8,10,14-eicosatetraenoate (1) (R_F=0.54). Ultraviolet spectrometry showed strong absorption with λ_{max}^{EtOH}=237 mµ indicating the

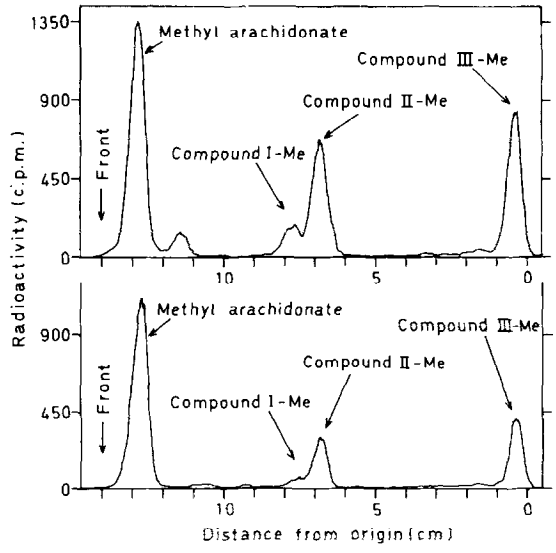

Fig. 1. Thin layer radiochromatograms of esterified products isolated after incubation of homogenates of guinea pig lung (2 g) in 6 ml of potassium phosphate buffer containing 24 µg of [1-^{14}C] arachidonic acid. Lower panel: 1 min at 37°; upper panel, 5 min at 37°. Solvent system: organic layer of ethyl acetate-2,2,4-trimethylpentane-water (50:100:100; v/v/v).

presence of one pair of conjugated double bonds. Gas-liquid chromatographic analysis showed that the trimethylsilyl (TMSi) derivatives of the two compounds had the same equivalent chain length (C-21.3; column, 1% OV-1 at 203°). Several mass spectra were recorded on the peak of the derivative of Compound I. Ions of high intensity were present at m/e 406 (M), 391 (M-15; loss of $\cdot CH_3$), 375 (M-31; loss of $\cdot OCH_3$), 335 (M-71; loss of $\cdot C_5H_{11}$ from 15-OH isomer), 316 (M-90; loss of TMSiOH), 295 (M-111; loss of $\cdot CH_2$-CH=CH-C_5H_{11} from 12-OH isomer), 229, 225 ($TMSiO^+$=CH-(CH=CH)$_2$-C_5H_{11} (11-OH isomer) and [(CH=CH)$_2$-CH(OTMSi)-C_5H_{11}]$^+$ (15-OH isomer), 205 (295-90), and 173 (mainly 295-(90+32)). The TMSi derivatives of methyl 15L-hydroxy-5,8,11,13-eicosatetraenoate (3) and methyl 12L-hydroxy-5,8,10,14-eicosatetraenoate (1) were used as references. The mass spectrum of the former derivative showed prominent ions at m/e 406, 391, 375, 335, 316, 225, and 173 whereas the mass spectrum of the latter derivative showed ions of high intensity at m/e 406, 391, 375, 295, 229, 205, and 173. Compound I was thus a mixture of three isomeric hydroxy-eicosatetraenoates, i.e. 12-hydroxy-5,8,10,14-eicosatetraenoic acid (about 70%), 11-hydroxy-5,8,12,14-eicosatetraenoic acid (about 26%) and 15-hydroxy-5,8,11,13-eicosatetraenoic acid (about 4%).

The methyl ester of Compound II cochromatographed with methyl 12L-hydroxy-5,8,10-heptadecatrienoate (1) (R_F=0.47). The ultraviolet spectra recorded on the two esters were identical (λ_{max}^{EtOH} =232 mμ). Gas-liquid chromatographic analysis of the TMSi derivatives of both compounds showed a peak with equivalent chain length corresponding to C-19.3. The mass spectra recorded on these peaks were identical and showed prominent ions at m/e 366 (M), 351 (M-15), 335 (M-31), 295 (M-71; loss of $\cdot C_5H_{11}$), 276 (M-90), and 225 (M-141; loss of $\cdot CH_2$-CH=CH-$(CH_2)_3$-$COOCH_3$) (1). On basis of these experiments Compound II was identified as 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT).

The methyl ester of Compound III was subjected to thin layer chromatography with benzene-dioxane (50:40, v/v) as solvent. A major peak (R_F=0.69, 80%) and two minor peaks (R_F=0.62, about 10%; R_F= 0.47, about 10%) appeared (references, methyl esters of PHD, R_F= 0.69; PGE_2, 0.62; $PGF_{2\alpha}$, 0.47). An aliquot of the major component was treated with 0.5 N NaOH at room temperature for 30 min. This did not result in formation of PGB_2 as shown by thin layer chromatography. Another part was treated with $NaBH_4$ in methanol. This

afforded a more polar derivative that migrated close to the methyl ester of $PGF_{2\beta}$ on thin layer chromatography. These experiments indicated that the parent acid of the major component differed from PGE_2, 8-iso-PGE_2, PGD_2 and other keto-prostaglandins but was identical with the hemiacetal derivative of 8-(1-hydroxy-3-oxo-propyl)-9,12-dihydroxy-5,10-heptadecadienoic acid (PHD) which compound was recently isolated after incubation of arachidonic acid with washed human platelets (1). This was conclusively proved by gas-liquid chromatographic-mass spectrometric analysis of the TMSi derivative of the methyl ester. The C-value was C-24.6 and the mass spectrum showed ions of high intensity at m/e 600 (M), 585 (M-15), 529 (M-71; loss of $\cdot C_5H_{11}$), 510 (M-90), 439 (529-90), 420 (M-2x90), 366 (M-234; elimination of propyl side chain with its substituents), 323, 301 (probably $TMSiO^+=CH-CH=CH-CH(OTMSi)-C_5H_{11}$), 295 (366-71), 256 (base peak, $[TMSiO-CH=CH-CH_2-CH=CH-(CH_2)_3-COOCH_3]^{+\cdot}$), 225 (366-141; loss of $\cdot CH_2-CH=CH-(CH_2)_3-COOCH_3$), 217 ($TMSiO^+=CH-CH=CH-$ -OTMSi), and 173 ($TMSiO^+=CH-C_5H_{11}$). Identical results were obtained with the TMSi derivative of the methyl ester of PHD (1). The less polar compound (R_F=0.62) of the two minor components was con-

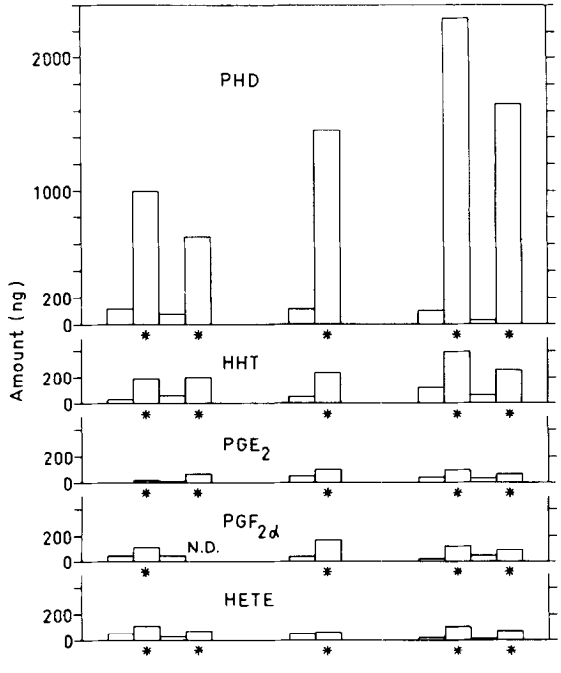

Fig. 2. Release of PHD, HHT, PGE_2, $PGF_{2\alpha}$, and HETE from perfused guinea pig lung. Asterisks indicate injection of 30 µg of arachidonic acid. N.D., not determined.

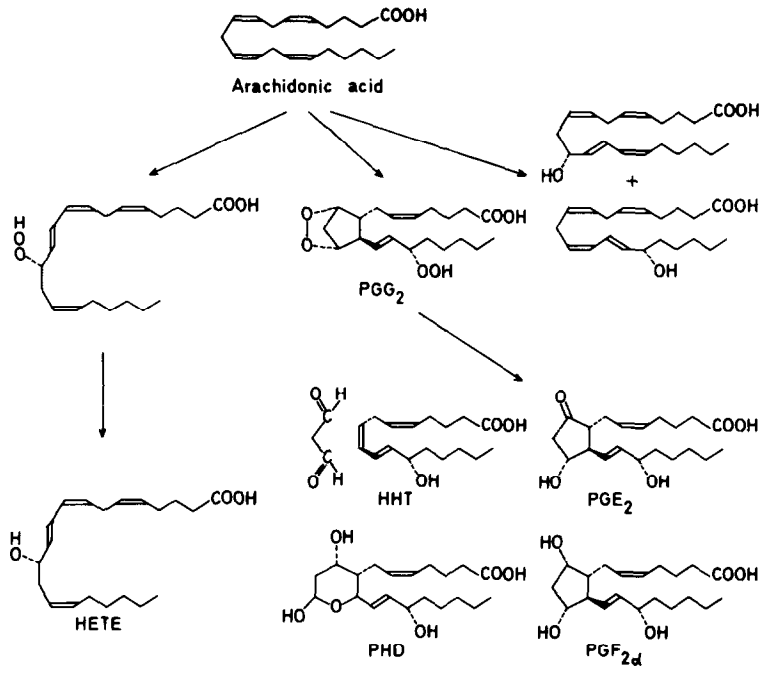

Fig. 3. Transformations of arachidonic acid in guinea pig lung.

verted into the O-methyloxime-TMSi derivative and subjected to gas-liquid chromatography-mass spectrometry. The more polar compound (R_F=0.47) was converted into the TMSi derivative and similarly analyzed. By using derivatives of authentic prostaglandins as references it could be established that the less polar compound was identical with the methyl ester of PGE_2 and the more polar compound with the methyl ester of $PGF_{2\alpha}$.

Perfused Guinea Pig Lung. Guinea pig lungs were perfused through the pulmonary artery with Krebs-Henseleit medium containing 0.2% glucose (flow rate, about 2 ml/min). The effluent was collected for periods of 10 min in flasks containing 150 ml of ethanol and then split into three equal parts. Two of these were used for quantitative determination of PGE_2 and $PGF_{2\alpha}$ using [3,3,4,4-$^2H_4$]PGE_2 and [3,3,4,4-$^2H_4$]$PGF_{2\alpha}$ as carriers (4). The remaining part was used for quantitative determination of PHD, HETE, and HHT using [5,6,8,9,11,12,14,15-$^2H_8$]PHD and [5,6,8,9,11,12,14,15-$^2H_8$]HETE (2). As shown in Fig. 2 the basal levels of prostaglandins and hydroxy acids in the lung perfusate were low. However, the levels rose sharply after injection of 30 µg of arachidonic acid into the pulmonary artery. In

five experiments with three different lungs the following amounts were released during the 10 min period after injection of arachidonic acid: PHD, 654-2304 ng; HHT, 192-387 ng; PGE_2, 15-93 ng; $PGF_{2\alpha}$, 93-171 ng; HETE, 66-111 ng.

DISCUSSION

Biosynthesis of prostaglandins by guinea pig lung was originally demonstrated by Änggård and Samuelsson (5). They found that when tritium-labeled arachidonic acid was incubated with the 900xg supernatant of a homogenate of guinea pig lung about 10% of the radioactivity was converted into products with chromatographic properties resembling prostaglandins. The major component was $PGF_{2\alpha}$ (about 5% yield from the labeled arachidonic acid) whereas PGE_2, 13,14-dihydro-PGE_2, and 15-keto-13,14-dihydro-PGE_2 were obtained in lower yields.

In the present work the transformations of [1-^{14}C]arachidonic acid by whole homogenates of guinea pig lung were studied. The formation of $PGF_{2\alpha}$ and PGE_2, both in 2-3% yield, was confirmed. However, the major component in the prostaglandin fraction (19% of the recovered radioactivity) was identical with the hemiacetal derivative of 8-(1-hydroxy-3-oxopropyl)-9,12-dihydroxy-5,10-heptadecadienoic acid (PHD). This compound migrated close to PGE_2 on thin layer chromatography but could be distinguished from this prostaglandin by its lack of conversion into PGB_2 by alkali and its conversion into a polar derivative different from $PGF_{2\alpha}$ and $PGF_{2\beta}$ by sodium borohydride. Several monohydroxy acids were also identified. 12L-Hydroxy-5,8,10-heptadecatrienoic acid (HHT) was isolated in about the same yield as PHD (21% of the recovered radioactivity) whereas 11-hydroxy-5,8,12,14-eicosatetraenoic acid, 15-hydroxy-5,8,11,13-eicosatetraenoic acid , and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE) were obtained in lower yields (1.6, 0.2, and 4.2%, respectively). HHT and PHD both originated in the prostaglandin endoperoxide, PGG_2. This was demonstrated by separate incubation of [1-^{14}C]PGG_2 (Hamberg, M., and Samuelsson, B., unpublished observations). 11-Hydroxy-5,8,12,14-eicosatetraenoic acid and 15-hydroxy-5,8,11,13-eicosatetraenoic acid and the corresponding analogous compounds formed from 8,11,14-eicosatrienoic acid have earlier been obtained as by-products during prostaglandin biosynthesis in the vesicular gland of sheep (6,7). They are probably formed by reduction of the corre-

sponding peroxy radicals or hydroperoxide derivatives which are believed to be formed during the conversion of the precursor acid into the endoperoxide intermediate. 12-Hydroxy-5,8,10,14-eicosatetraenoic acid was recently isolated in high yield after incubation of arachidonic acid with washed human platelets (1). This acid was formed by reduction of the corresponding hydroperoxide which was in turn formed from arachidonic acid by a fatty acid dioxygenase different from that involved in prostaglandin biosynthesis.

The experiments with perfused guinea pig lung corroborated the findings with homogenates in that large amounts of PHD were released following injection of arachidonic acid. The remaining products including PGE_2 and $PGF_{2\alpha}$ were obtained in lower yield. It is noteworthy that in both systems added arachidonic acid was to a considerably greater extent converted into the endoperoxide metabolites, PHD and HHT, than to the classical prostaglandins. The same situation was recently encountered in a study on prostaglandin biosynthesis during thrombin-induced aggregation of washed human platelets. In this case the total synthesis of PGG_2 as judged from the sum of PHD and HHT was approximately two orders of magnitude higher than that of PGE_2 and $PGF_{2\alpha}$ (2).

The efficient conversion of added arachidonic acid into PGG_2 by platelets and guinea pig lung is intriguing in view of the pronounced biological effects exerted by PGG_2 (and PGH_2) on these tissues. Platelets undergo irreversible aggregation in the presence of PGG_2 and PGH_2 and a role of endogenous endoperoxides in platelet aggregation has been proposed (8,2). The endoperoxides have pronounced stimulatory effect on respiratory smooth muscle *in vitro* and *in vivo* (9) and the possible role of endoperoxides in causing the bronchoconstriction of anaphylaxis and other pathological states should be investigated.

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