FORMATION OF 6-OXOPROSTAGLANDIN $F_{1\alpha}$ BY ARTERIES OF THE FETAL CALF

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Received March 1,1977

SUMMARY

Homogenates from fetal calf aorta converted arachidonic acid to two main products which were shown by gas chromatography-mass spectrometry to be different forms of 6-oxoprostaglandin $F_{1\alpha}$. Aortas from fetal calves of all gestational ages investigated (100 to 240 days of gestation) as well as ductus arteriosus and brachiocephalic artery were active in converting arachidonic acid to these products.

INTRODUCTION

Prostaglandins (PG's) or related compounds appear to play a role in maintaining the patency of the ductus arteriosus during gestation (1-3). Little is known concerning the biosynthesis of prostaglandins in this tissue or in fetal aorta. For these reasons we investigated the metabolism of arachidonic acid in fetal aorta and ductus arteriosus. It would appear that the major pathway of arachidonic acid metabolism in these tissues has 6-oxoPG$F_{1\alpha}$ as its end product.

METHODS

Unlabeled arachidonic acid and [1-^{14}C]arachidonic acid (55 Ci/mole) were purchased respectively from Nu Chek Prep, Inc.,

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Abbreviations: PG, prostaglandin; TLC, thin-layer chromatography; GC-MS, gas chromatography-mass spectrometry; TMS, trimethylsilyl.

ISSN 0006-291X

Elysian, Minn. and the Radiochemical Center, Amersham. [5,6,8,9,11,12,14,15-^{2}H]Arachidonic acid was prepared from 5,8,11,14-eicosatetraynoic acid by the method of Hamberg et al (4).

Samples (0.2 g) of fetal calf aorta, ductus arteriosus or brachiocephalic artery were homogenized with a ground glass homogenizer in 5 volumes of 0.05 M Tris-HCl, pH 7.4, containing [1-^{14}C]-arachidonic acid (0.15 μCi). The mixtures were incubated in a water bath at 37°C for 15 min and the incubations terminated with ethanol (2 volumes). The mixtures were filtered and the filtrates concentrated in vacuo. After acidification with 1 N HCl to pH 3 and extraction with ether, the extracted products were methylated with diazomethane. The products were analyzed by thin-layer chromatography (TLC) using plastic sheets precoated with silica gel F-254 (EM Reagents, Darmstadt) with 4% methanol in ether as solvent. The plates were scanned with a Packard model 7200 radiochromatogram scanner and the radioactive bands eluted with methanol/ether (1:1).

Purification of 6-oxo$PGF_{1\alpha}$: Fetal calf aorta (9 g) was homogenized with a Virtis homogenizer in 5 volumes of 0.05 M Tris, pH 7.4 containing [1-^{14}C]arachidonic acid (1.5 μCi) and either arachidonic acid (1 mg) or [5,6,8,9,11,12,14,15-^{2}H]arachidonic acid (1 mg). The mixture was shaken at 37°C for 40 min, and ethanol (2 volumes) was then added. The filtrate was concentrated in vacuo, acidified to pH 3 with HCl and extracted with ether. After evaporation of the ether, the residue was chromatographed on an LH-20 column (17 cm x 1 cm) and eluted with benzene/dichloromethane/methanol (10:10:1). The fraction containing the radioactive products was concentrated to dryness in vacuo. After methylation with diazomethane, the products were purified by TLC on silica gel G with 1.5% methanol in ether as solvent.

RESULTS

Incubation of homogenates of fetal calf aorta with [1-^{14}C]-arachidonic acid (4 μg/g tissue) for 15 min at 37°C gave 2 main radioactive products (Fig. 1A). The most abundant product (17% of recovered radioactivity) had an R_f value (after methylation) of 0.46 in 4% methanol/ether. The second product (5% of recovered radioactivity) had an R_f value identical to that of PGE_2 methyl ester (R_f 0.39). Addition of indomethacin (10^{-5}M) to the incubation medium inhibited the formation of these compounds, suggesting that fatty acid cyclo-oxygenase was required for their formation. These products were not identical to PGE_2, $PGF_{2\alpha}$ or

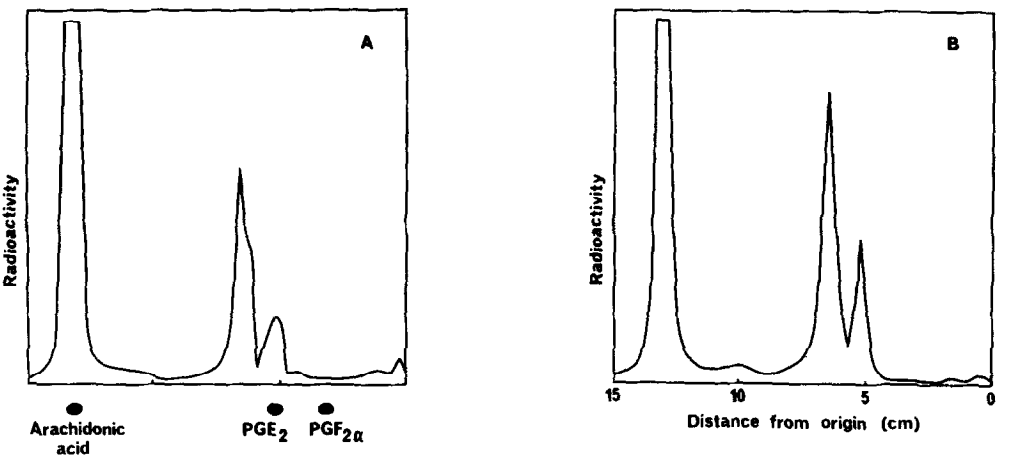

Fig. 1. Thin-layer radiochromatograms of the ether extractable products obtained after incubation of [1-^{14}C] arachidonic acid (0.15 µCi) with homogenates of fetal calf aorta (0.2 g) (A) and ductus arteriosus (0.2 g) (B) at 37C for 15 min. Solvent system: 4% methanol in ether.

thromboxane B_2 since quantitation by GC-MS indicated that only small amounts of the latter compounds were formed by homogenates of aorta (5).

Homogenates of fetal calf aortas of all the gestational ages investigated (100 to 240 days of gestation; term is 265 days) were all quite active in converting arachidonic acid to the two products described above. The chromatographic patterns obtained after incubation of homogenates of fetal calf ductus arteriosus (Fig. 1B) and brachiocephalic artery with [1-^{14}C]-arachidonic acid were identical to those obtained from aorta. The 100,000 x g pellet from fetal calf aorta (1.2 g) also converted arachidonic acid (10 µg/g of tissue) to the products with R_f values of 0.39 (7%) and 0.46 (17%) after incubation at 37°C

for 40 min. This indicates that the enzymes responsible for their formation are particle bound and that cytosol factors are not required.

In order to identify the major biosynthetic products [1-^{14}C]-arachidonic acid (1.5 μCi) and unlabeled arachidonic acid (1 mg) were incubated with fetal calf aorta for 40 min at 37°C. The products were purified by column chromatography on LH-20 and, after methylation, by TLC on silica gel G with 1.5% methanol in ether as solvent. The resulting two bands of radioactive material (R_f values 0.15 and 0.22) were eluted and the products analyzed by GC-MS after conversion to their trimethylsilyl (TMS) or O-methyloxime-TMS derivatives.

The mass spectrum of the minor product (R_f 0.15) as its TMS derivative (retention time, 5.8 min on a 40 cm column of 0.5% OV-101) was identical to that reported by Johnson et al (6) for the TMS derivative of 6-oxoPGF$_{1\alpha}$ methyl ester. The mass spectrum of the O-methyloxime-TMS derivative of this compound (retention time, 5.3 min) was identical to that reported for the TMS derivative of 6-oxoPGF$_{1\alpha}$ methyl ester, O-methyloxime by Pace-Asciak (7). The mass spectrum of the corresponding product (methyl ester, TMS) formed from [5,6,8,9,11,12,14,15-^{2}H]arachidonic acid showed the expected shifts at m/e 536(7D), 517(7D), 516(6D), 446(7D), 445(6D), 426(6D), 425(5D), 355(6D), 328(4D), 282(5D), 281(4D), 219(2D), 201(2D), 192(1D), 174(1D), 144(1D). These results indicate that the product with an R_f value of 0.15 is 6-oxoPGF$_{1\alpha}$ methyl ester.

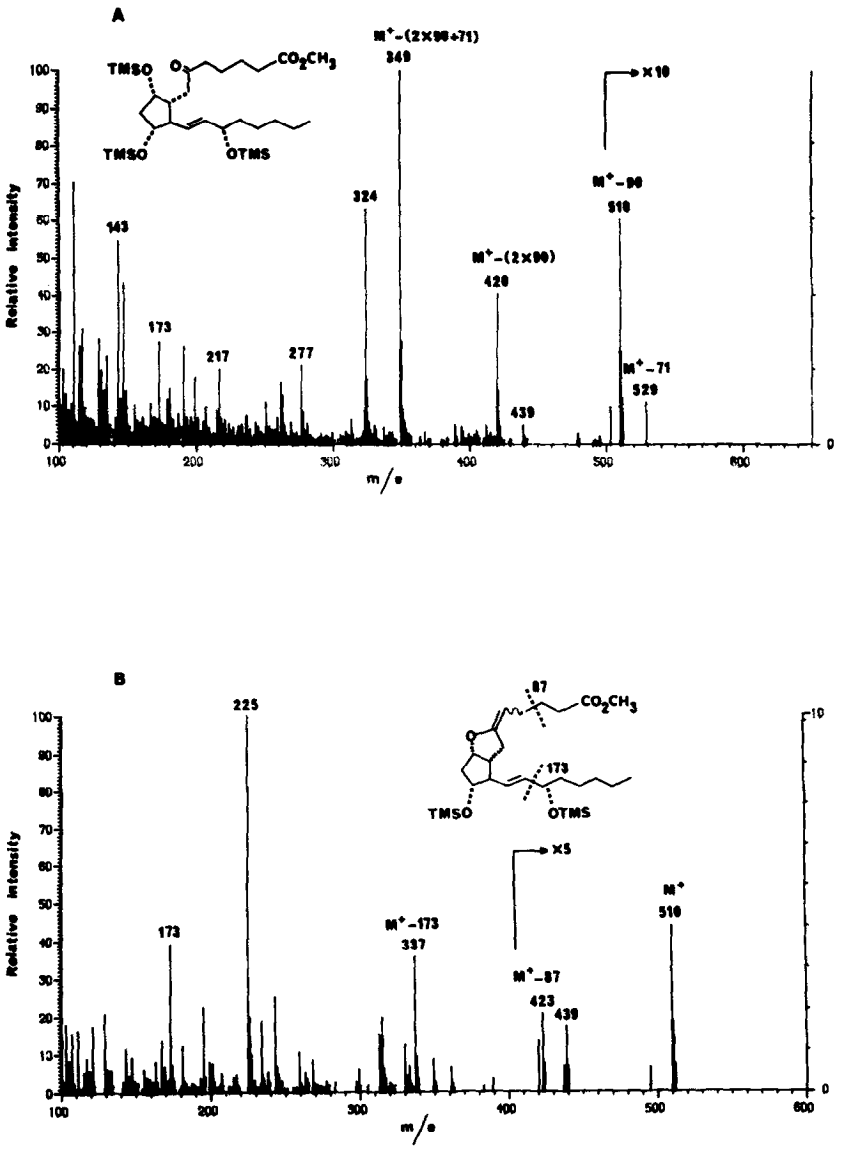

Fig. 2. Mass spectra of the products obtained after trimethylsilylation of 2 products isolated after incubation of arachidonic acid with a fetal calf aorta homogenate. A, minor product, R_f of methyl ester, 0.15 with 1.5% methanol in ether as solvent (silica gel G) and 0.39 with 4% methanol in ether as solvent (silca gel F-254; cf. Fig. 1). B, major product, R_f of methyl ester, 0.22 with 1.5% methanol in ether as solvent (silica gel G) and 0.46 with 4% methanol in ether as solvent (silica gel F-254; cf. Fig. 1).

The mass spectrum of the O-methyloxime-TMS derivative of the major product (R_f 0.22) was also identical to that of the TMS derivative of 6-oxo$PGF_{1\alpha}$ methyl ester, O-methyloxime. The TMS derivative of this product had a retention time of 3.6 min and a mass spectrum (Fig. 2B) identical to that reported by Johnson et al (6) for the TMS derivative of 9-deoxy-6,9α-epoxy-Δ^5-$PGF_{1\alpha}$ methyl ester. This compound was thought to be formed by loss of trimethylsilanol after trimethylsilylation of the lactol form of 6-oxo$PGF_{1\alpha}$ methyl ester (6).

These results indicate that both of the products isolated from fetal calf aorta homogenates are forms of 6-oxo$PGF_{1\alpha}$. The major product would appear to be a lactol form of this compound. The minor product could be either the lactol form with the opposite configuration at the 6-carbon, or the uncyclized keto form.

DISCUSSION

The formation of 6-oxo$PGF_{1\alpha}$ has recently been shown to be the major pathway of PGH_2 metabolism by microsomes from adult pig aorta (6). 6-Oxo$PGF_{1\alpha}$ is formed from an unstable substance which was named prostacyclin, the structure of which is 9-deoxy-6,9α-epoxy-Δ^5-$PGF_{1\alpha}$ (6). Prostacyclin relaxes vascular smooth muscle (8) and is very potent in inhibiting platelet aggregation (9). Evidence for the formation of a substance with the structure of prostacyclin was first reported in rat stomach homogenates by Pace-Asciak and Wolfe (10). Guinea pig lungs (11) and rat granuloma tissue (12) have also recently been reported to synthesize 6-oxo$PGF_{1\alpha}$.

It is interesting that both fetal calf aorta homogenates and particulate fractions are very active in converting arachidonic acid to 6-oxo$PGF_{1\alpha}$, presumably via prostacyclin. This substrate is not converted to prostacyclin by adult pig or rabbit aorta microsomes (9). Adult rabbit aortic rings converted arachidonic acid to prostacyclin in low yield (0.5-1%) (8).

It is possible that intermediates in the formation of 6-oxo$PGF_{1\alpha}$, such as prostacyclin, could have a special function in the fetus. The fact that this appears to be the major pathway of arachidonic acid metabolism in the ductus arteriosus is interesting in view of the role of prostaglandins in maintaining the patency of the ductus throughout gestation. It is possible that prostacyclin may have an important role to play in this process.

ACKNOWLEDGEMENTS

We are grateful to Dr. O. Mamer for assistance with the mass spectrometry, to Dr. J.E. Pike of the Upjohn Co. for prostaglandin standards, to Dr. J.R. Paulsrud of the Hoffman-LaRoche Co. for 5,8,11,14-eicosatetraynoic acid and to Dr. L.S. Wolfe for helpful discussions. The technical assistance of Mrs. J. Landreville and Mrs. A.M. Pietromonaco is gratefully acknowledged. The research was supported by grants from the MRC of Canada and the U.S. P.H.S. and the Conseil de la recherche en santé du Québec.

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