Journal of Chromatography, 394 (1987) 353–362
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 440

QUANTITATIVE DETERMINATION OF PROSTAGLANDINS E_1, E_2 AND E_3 IN FROG TISSUE

CEIL A. HERMAN*,*, MATS HAMBERG and ELISABETH GRANSTRÖM
Department of Physiological Chemistry, Karolinska Institute, S-104 01 Stockholm (Sweden)
(First received September 22nd, 1986; revised manuscript received January 14th, 1987)

SUMMARY

A method was developed for quantitative determination of endogenous production of prostaglandin (PG)E_1, PGE_2 and PGE_3 by *Rana temporaria* lung, heart and urinary bladder homogenates, since these tissues contain the precursors, 8,11,14-eicosatrienoic, arachidonic and 5,8,11,14,17-eicosapentaenoic acids. Following homogenization and shaking at 22°C for 30 min, media were extracted by XAD-2, treated with sodium hydroxide in order to convert PGE compounds into PGB compounds, purified by thin-layer chromatography, and analyzed by high-performance liquid chromatography with homo-PGE_1 as an internal standard. The ratio of prostaglandins E_1, E_2 and E_3 compared to the ratio of fatty acid precursors in tissue suggested that the tissue content of precursor is not the only factor determining the type of prostaglandin synthesized.

INTRODUCTION

Prostaglandin synthesis from endogenous and exogenous precursors has been extensively studied in mammalian tissues[1–4], but little is known of endogenous prostaglandin synthesis in non-mammalian vertebrates. While mammals produce dienoic prostaglandins utilizing arachidonic acid ($C_{20:4}$, $\omega - 6$) as substrate[1,2,4], studies of the fatty acid composition of tissue lipids in frogs show that, unlike mammals, membranes contain not only arachidonic acid, but also eicosapentaenoic acid ($C_{20:5}$, $\omega - 3$), and eicosatrienoic acid ($C_{20:3}$, $\omega - 6$), the precursors for the trienoic and monoenoic prostaglandins, respectively[5,6]. Therefore, the potential exists in frogs for production of prostaglandins from different fatty acid precursors.

Exogenous fatty acid precursors have been shown to have effects in amphibians[6–10], presumably due to their conversion to biologically active products. *In vitro* studies using *Rana pipiens* urinary bladder showed opposite effects of arachidonic and eicosapentaenoic acid derived metabolites on water permeability[6,7]. Exogenous PGE_2 and arachidonic acid both inhibited water flow, while PGE_3 and eicosapen-

* Present address: Department of Biology, Box 3AF, New Mexico State University, Las Cruces, NM 88003, U.S.A.

0021-9673/87/$03.50

taenoic acid stimulated water flow. The effects of the exogenous precursors could be attenuated with indomethacin. *In vivo* studies using cannulated *Rana catesbeiana* showed that infused PGE_1, PGE_2 and PGE_3 or their precursors, eicosatrienoic, arachidonic and eicosapentaenoic acids respectively all resulted in hypotension and the effect of the precursors was attenuated with indomethacin[8–10].

Prostaglandin synthesis studies in non-mammalian vertebrates have been carried out using exogenous substrates[11–14] or tissue extracts[15]. In studies examining the conversion of [1-^{14}C]arachidonic acid by several invertebrate and vertebrate species, the vertebrates produced more PGE_2 as measured by thin-layer chromatography (TLC)[11]. Tissues from several species were examined for their ability to convert [^{3}H]eicosatrienoic acid (10 μg) into PGE_1 and $PGF_{1\alpha}$[12]. Frog (Rana) urinary bladder converted 17% of the eicosatrienoic acid into PGE_1 measured as PGB_1 at 278 nm following alkaline treatment while conversion by frog lung was 10%[12]. Toad urinary bladder (*Bufo marinus*) converted [1-^{14}C]arachidonic acid to PGE_2 and $PGF_{2\alpha}$ as measured by TLC[13]. Isolated, perfused frog hearts (*Rana esculenta*) produced basal quantities of PGE_2, TXB_2 and 6-keto $PGF_{1\alpha}$ as detected by radioimmunoassay, and the production was stimulated with exogenous unlabelled arachidonic acid[14]. Tissue contents of PGE_2 from frog (*Rana nigromaculata*) tissues was greatest in the gastrointestinal tract and lowest in liver of the tissues examined, as measured by bioassay[15].

Studies with exogenous substrate may not accurately reflect endogenous product formation. Analysis of monoenoic, dienoic and trienoic prostaglandins has been difficult due to the low amounts of compounds that are produced and the lack of discrimination of current radioimmunoassays for the prostaglandins produced from different 20 carbon precursors. The purpose of this study was to develop a method which would allow the quantitation of PGE_1, PGE_2 and PGE_3 produced by frog tissues. The representative tissues chosen for analysis were lung, heart, and urinary bladder.

EXPERIMENTAL

Animals

Rana temporaria were obtained locally or from the Stockholm Biological Laboratory, Stockholm, Sweden. The frogs were housed in large tanks with water (22°C) under controlled light conditions (12 h light/12 h dark). They were force-fed pig heart three times a week.

Materials

PGE_3 was a kind gift from Ulf Diczfalusy, Department of Clinical Chemistry, Huddinge University Hospital, Huddinge, Sweden. PGE_1, PGE_2 and XAD-2 were purchased from Sigma, St. Louis, MO, U.S.A. BSTFA [bis(trimethylsilyl)trifluoroacetamide], polyunsaturated fatty acid mixtures, boron trifluoride in methanol, and SP-2330 (10%) on Supelcoport (100–120 mesh) were obtained from Supelco, Bellafonte, PA, U.S.A. [1-^{14}C]Arachidonic acid (59.3 mCi/mmol), [1-^{14}C]-5,8,11,14,17-eicosapentaenoic acid (56.9 mCi/mmol), [1-^{14}C] 8,11,14-eicosatrienoic acid (56.0 mCi/mmol) and [5,6,8,11,12,14,15-^{3}H]prostaglandin E_2 (200 Ci/mmol) were obtained from New England Nuclear, Boston, MA, U.S.A. Sep-Pak C_{18} car-

tridges were obtained from Waters Assoc., Milford, MA, U.S.A. Silica gel G TLC plates (250 μm, without fluorescent indicator) were purchased from Merck, Darmstadt, F.R.G.

Analysis of tissue fatty acids

Pooled frog lung, heart and urinary bladder tissues (approximately 200 mg per sample) were homogenized with a polytron and saponified in potassium hydroxide and methanol as previously described[16]. Following reflux for 1 h, samples were acidified to pH 3.0, extracted twice with diethyl ether, and evaporated to dryness with nitrogen. Samples were methylated with boron trifluoride in methanol[10]. The analysis for fatty acid composition was made using a Hewlett-Packard gas chromatograph, Model 5710A equipped with a splitless inlet, a flame ionization detector, and a 2.5 m × 2 mm I.D. glass column containing 1.4 g of SP-2330 (10%) on Supelcoport (100–200 mesh). Gas chromatographic conditions were as follows: oven temperature 175°C (4 min), then increased to 220°C at the rate of 8°C/min. Detector and injector port temperatures were 250°C, and the nitrogen flow-rate was 30 ml/min. Standard mixtures of methylated polyunsaturated fatty acid standards were used and calculation of the percent fatty acid in the total mixture was made by integration of peaks in the chromatogram using a Hewlett-Packard 3390A integrator. The data represent mean ± S.E.M. of 4 determinations.

Two-dimensional TLC

Frog lung, heart and urinary bladder (250 mg/ml 0.1 *M* phosphate buffer, pH 7.4) were homogenized with a polytron and incubated with [1-^{14}C]arachidonic acid (100 000 cpm) at 22°C for 30 min. The media were adjusted to pH 3.0 and the samples were purified with Sep-Pak C_{18} cartridges[17]. The methyl formate fraction was analyzed by TLC on silica gel plates using diethyl ether–acetic acid (100:2) in the first dimension and chloroform–methanol–acetic acid (90:10:2) in the second dimension. Prostaglandin standards were visualized with phosphomolybdate spray reagent and heating. Radioactive spots were visualized by autoradiography[18].

One-dimensional TLC

Lung tissue was homogenized using a polytron (250 mg/ml 0.1 *M* phosphate buffer, pH 7.4) and incubated with [1-^{14}C]arachidonic acid (2 000 000 cpm) at 22°C for 30 min. Medium was adjusted to pH 3.0 and extracted twice with diethyl ether. Samples were esterified by reaction with an excess of diazomethane in diethyl ether for 2 min at room temperature and analyzed by TLC using silica gel plates and diethyl ether–methanol (98:2) as solvent. Control incubations and those containing indomethacin (0.1 m*M*) were compared. The zone corresponding to the authentic methyl PGE_2 standard in the control incubation was scraped from the plate and the silica gel eluted with 1 ml methanol. The sample was then treated with 15 mg sodium borohydride for 30 min at room temperature and subjected to re-chromatography on silica gel plates using diethyl ether–methanol (95:5) as solvent to confirm the identity of the radioactive zone as methyl PGE_2 by its conversion to methyl $PGF_{2\alpha}$ and methyl $PGF_{2\beta}$.

Synthesis of homo-prostaglandin E_1

Homo-prostaglandin E_1 for use as an internal standard was prepared as previously described by (i) one-carbon elongation of 8,11,14-eicosatrienoic acid into 9,12,15-heneicosatrienoic acid followed by (ii) incubation of 9,12,15-heneicosatrienoic acid with the microsomal fraction of homogenates of the sheep vesicular gland[19]. The homo-PGE_1 was purified by silicic acid chromatography[19] and high-performance liquid chromatography (HPLC) using a 200 × 4.6 mm reversed-phase C_{18} column (Nucleosil 5, Alltech) monitored at 205 nm with a mobile phase of acetonitrile–water [36:64 (v/v) containing 0.1% acetic acid to give an apparant pH of 3.7]. The injection volume was 100 μl containing 100 μg homo-PGE_1. The flow-rate was 0.5 ml/min and the retention time for homo-PGE_1 was 19 min.

Quantitation of PGE compounds by HPLC

Frog lung, heart and urinary bladder tissues were homogenized with a polytron (250 mg/ml in 0.1 *M* phosphate buffer, pH 7.4) and incubated at 22°C for 30 min. Following the incubation, homo-prostaglandin E_1 (1275 ng) was added as an internal standard. The incubation medium was diluted to 10 ml, centrifuged at 750 *g*, the pH adjusted to 3.0, and applied to a 25 × 2 cm glass column containing 25 ml XAD-2 with a bed height of 9.5 cm. The column was washed with 90 ml distilled water, and then eluted with 80 ml ethanol. The ethanol fraction containing the PGE compounds was evaporated, redisolved in 1 ml ethanol, and subjected to treatment with 1 ml 0.5 *M* sodium hydroxide for 20 min. The reaction mixture was acidified to pH 3.0 and extracted twice with diethyl ether. The diethyl ether was evaporated and the samples disolved in ethyl acetate and purified by preparative TLC on silica gel plates using diethyl ether–methanol–acetic acid (98:2:0.5) as solvent and [^{3}H]PGB_2 (9000 cpm) as standard. The zone corresponding to PGB was determined by scanning the plate to determine the location of the standard. The zone was scraped and the silica gel eluted with 5 washes of 10 ml methanol containing 1% acetic acid. The solvent was centrifuged at 750 *g* to remove the silica gel, evaporated, and redisolved in the mobile phase (200 μl) for HPLC. Analysis was carried out using a 200 × 4.6 mm reversed-phase C_{18} column (Nucleosil 5, Alltech) monitored at 280 nm and a mobile phase of acetonitrile–water (40:60, v/v) containing 1% acetic acid, to give an apparent pH of 3.7[20]. The typical injection volume ranged between 10 and 100 μl. Authentic PGB_1, PGB_2 and PGB_3 standards were prepared from PGE_1, PGE_2 and PGE_3, respectively, by treatment with sodium hydroxide as described above for determination of retention times. [^{3}H]PGB_2 was prepared from [^{3}H]PGE_2 for TLC standard. The structures of the PGB standards were verified by mass spectrometry (MS).

MS of compounds from HPLC

Samples were collected from the HPLC, esterified by treatment with an excess of diazomethane in diethyl ether for 2 min, and further purified by preparative TLC on silica gel plates, using diethyl ether–methanol (98:2) as a solvent. They were eluted from the plate with ethyl acetate, and the trimethylsilyl derivative prepared using BSTFA (25 μl) and pyridine (10 μl). The samples were evaporated and dissolved in hexane. Mass spectrometry was performed on a 25 m × 0.32 mm I.D. (SPB-1, Supelco) capillary column on a Finnigan Model 4500 mass spectrometer. The oven temperature was 250°C and the ion source temperature was 190–200°C. The energy

of the ionization beam was maintained at 70 eV. A standard fatty acid methyl ester mixture was used for the conversion of retention times to *C* values.

RESULTS

Table I shows the fatty acid profile for lung, heart and urinary bladder tissues. The principle prostaglandin precursor found in all three frog tissues was arachidonic acid (6.5 ± 1.0 to 9.4 ± 0.9% of the total fatty acid composition). Eicosapentaenoic acid ranged from 1.7 ± 0.1 to 4.5 ± 1.0%, and eicosatrienoic acid was the least abundant precursor (0.6 ± 0.1 to 1.6 ± 0.3%).

Studies using [1-^{14}C]arachidonic acid analyzed by two-dimensional TLC and autoradiography demonstrated that the primary product of cyclooxygenase metabolism was a compound migrating with the authentic PGE_2 standard. Fig. 1 shows a tracing of the prostaglandin standards on the TLC plate with the X-ray film placed over the TLC plate. The figure shows results from an incubation of frog lung, however heart and urinary bladder demonstrated identical profiles. In all cases, only one radioactive spot was observed and it co-migrated with the authentic PGE_2 standard. Likewise, in other experiments when [1-^{14}C]eicosatrienoic or [1-^{14}C]eicosapentaenoic acids (100 000 cpm) were incubated with these tissues, one major radioactive spot was observed, co-migrating with PGE_2 standard (data not shown).

When lung tissue was incubated with [1-^{14}C]arachidonic acid followed by methylation of the compounds in the reaction mixture and analysis by one-dimensional TLC using a different solvent system, one principal radioactive product was produced which co-migrated with the authentic methyl PGE_2 standard (Fig. 2a). This peak could be abolished by the inclusion of 0.1 m*M* indomethacin in the in-

TABLE I

FATTY ACID DISTRIBUTION IN LIPIDS OF FROG TISSUES

Rana temporaria tissues (approximately 200 mg) were extracted and fatty acid composition was determined by gas chromatography as described in Experimental. (n = 4).

Carbon No.	*Mean percent of total fatty acid ± S.E.M.**		
	Lung	*Heart*	*Urinary bladder*
14:0	2.0 ± 0.3	2.1 ± 0.2	1.1 ± 0.5
16:0	16.2 ± 2.0	12.3 ± 0.3	16.7 ± 4.2
16:1	10.3 ± 1.9	12.2 ± 4.0	8.8 ± 0.9
18:0	5.8 ± 1.3	3.5 ± 0.6	5.2 ± 2.5
18:1	22.1 ± 1.8	20.7 ± 3.3	24.7 ± 1.9
18:2	9.7 ± 1.0	10.1 ± 1.7	8.9 ± 0.8
20:3	1.6 ± 0.3	0.7 ± 0.1	0.6 ± 0.1
20:4	9.4 ± 1.0	6.5 ± 1.0	8.7 ± 0.2
20:5	4.5 ± 1.0	2.0 ± 0.4	1.7 ± 0.1
22:4	1.4 ± 0.4	2.0 ± 0.1	3.9 ± 1.9
22:5	1.0 ± 0.4	0.7 ± 0.2	0.9 ± 0.3
22:6	0.8 ± 0.1	1.7 ± 0.1	0.9 ± 0.2

* Fatty acid percentages do not sum to 100%. The differences represent minor or unidentified peaks.

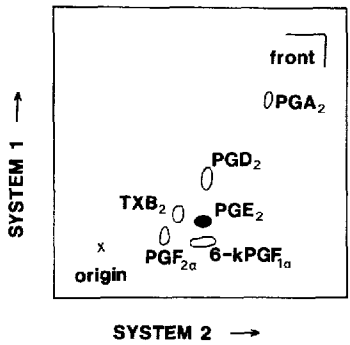

Fig. 1. Frog lung incubation with [1-^{14}C]arachidonic acid with results shown on two-dimensional TLC. Authentic prostaglandin standards were visualized with phosphomolybdate spray reagent and heating and are shown as open circles. Overlay of X-ray film showed one major radioactive spot which co-migrated with the PGE_2 standard (dark circle).

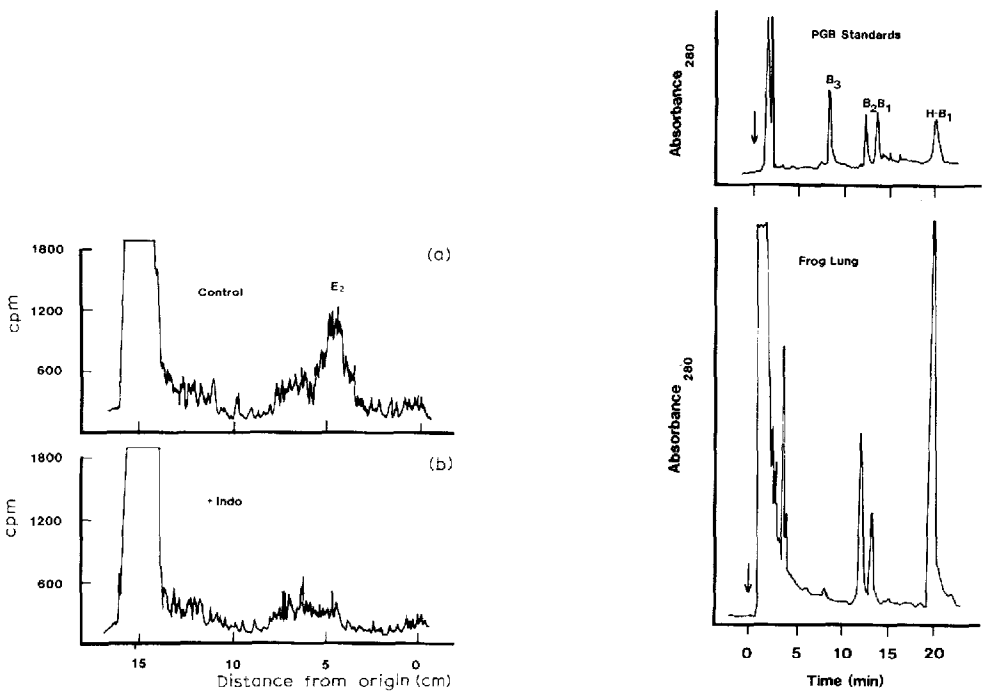

Fig. 2. Frog lung incubation with [1-^{14}C]arachidonic acid with results shown on one-dimensional thin-layer radiochromatography. (a) One major radioactive peak co-migrated with the authentic PGE_2 standard, visualized with phosphomolybdate spray and heating. (b) Absence of radiolabelled peak in the presence of 0.1 m*M* indomethacin.

Fig. 3. Quantitation of PGE compounds in frog lung by HPLC on Nucleosil 5-C_{18}. Other conditions: see text. PGE compounds were treated with 0.5 *M* sodium hydroxide and analyzed as PGBs. Retention times of standards PGB_3, PGB_2, PGB_1 and homo-PGB_1 were 8.3, 12.2, 13.8 and 20.0 min, respectively.

TABLE II

QUANTITATION OF PGE_1, PGE_2 AND PGE_3 IN FROG TISSUES

Rana temporaria tissues (250 mg/ml) were homogenized and incubated for 30 min at 22°C. Homo-PGE_1 (1275 ng) was added as an internal standard. Incubation media was extracted by XAD-2 and treated with 0.5 *M* sodium hydroxide to convert E to B compounds. The media were purified by preparative TLC and PGB compounds quantitated by HPLC as described in Experimental. Results represent mean ±S.E.M. of 4 determinations.

	pg prostaglandin/mg wet weight/30 min		
	PGE_1	*PGE_2*	*PGE_3*
Lung	1731.0 ± 101.6	3619.4 ± 288.9	192.0 ± 24.8
Heart	723.0 ± 150.0	1515.5 ± 225.7	47.3 ± 1.0
Urinary bladder	772.0 ± 70.0	7444.0 ± 840.0	263.0 ± 20.1

cubation media (Fig. 2b). Furthermore, the radioactive peak eluted from the TLC plate, when reduced with sodium borohydride resulted in the generation of two peaks which co-migrated with methyl $PGF_{2\alpha}$ and methyl $PGF_{2\beta}$.

Fig. 3 shows a representative tracing of a lung tissue analysis in which the principle E prostaglandin detected as PGB is PGE_2. The amount of PGE_1 was approximately half of the PGE_2. Smaller quantities of PGE_3 were detected. The proportions of PGE_1, PGE_2 and PGE_3 measured were dependent on the tissue, and results for lung, heart and urinary bladder are presented in Table II. However, PGE_2 was always the major E prostaglandin produced. PGE_2 and PGE_3 production was greatest in urinary bladder while the lung produced the greatest quantity of PGE_1. When urinary bladder was incubated whole, prostaglandins produced were approximately half of those shown for the homogenized tissue. In separate studies using the same tissues from *Xenopus laevis*, PGE_2 was also the principal PGE compound produced (data not shown).

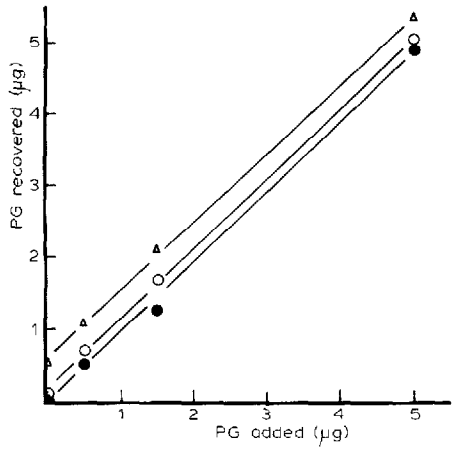

Fig. 4. Recovery of added prostaglandin E_1 (○), E_2 (△) and E_3 (●). Pooled frog lung was divided into samples containing 130 mg and incubated at 22°C for 30 min. PGE_1, PGE_2 and PGE_3 were added in quantities of 0.5, 1.5 and 5.0 μg following the incubation and samples were analyzed as described in Experimental.

To test the recovery of added prostaglandins, a large pool of frog lung was homogenized and equally subdivided to contain 130 mg of lung tissue in each sample. Following the 30-min incubation, PGE_1, PGE_2 and PGE_3 at concentrations of 0.5, 1.5 and 5.0 μg were added. Fig. 4 shows the results of this recovery experiment. Recovered amounts of PGE_1, PGE_2 and PGE_3 were the same as added amounts of prostaglandins plus endogenous levels. Without addition, lung tissue contained 225.0 ± 12.4, 470.5 ± 34.5 and 25.0 ± 3.2 ng of PGE_1, PGE_2 and PGE_3, respectively (n = 4, mean ± S.E.M.).

Fig. 5a shows the mass spectrum of the trimethylsilyl derivative (C=24.0) of the compound from lung tissue co-eluting with the PGB_2 standard on HPLC. The mass spectrum showed ions of high intensity at m/e 420 (M), 349 (M − 71; loss of $\cdot C_5H_{11}$), 321 (M − 99: loss of $\cdot C_5H_{11}$ plus CO from the five-membered ring), 299 [M − (90 + 31); loss of $(CH_3)_3SiOH$ plus $\cdot OCH_3$], 279 [M − 141; loss of $\cdot CH_2$–CH=CH–$(CH_2)_3$–$COOCH_3$] and 247 [M − 173, loss of $(CH_3)_3SiO$=CH–$(CH_2)_4$–CH_3]. An identical mass spectrum was obtained from the peak from urinary bladder which co-eluted with the PGB_2 standard.

Fig. 5b shows the mass spectrum of the trimethylsilyl derivative (C=24.0) of the compound from lung tissue co-eluting with the PGB_1 standard on HPLC. The mass spectrum showed ions of high intensity at m/e 422 (M), 351 (M − 71; loss of $\cdot C_5H_{11}$), 323 (M − 99; loss of $\cdot C_5H_{11}$ plus CO from the five-membered ring), 301 [M − (90 + 31); loss of $(CH_3)_3SiOH$ plus $\cdot OCH_3$] and 249 [M − 173; loss of $(CH_3)_3$ SiO=CH–$(CH_2)_4$–CH_3].

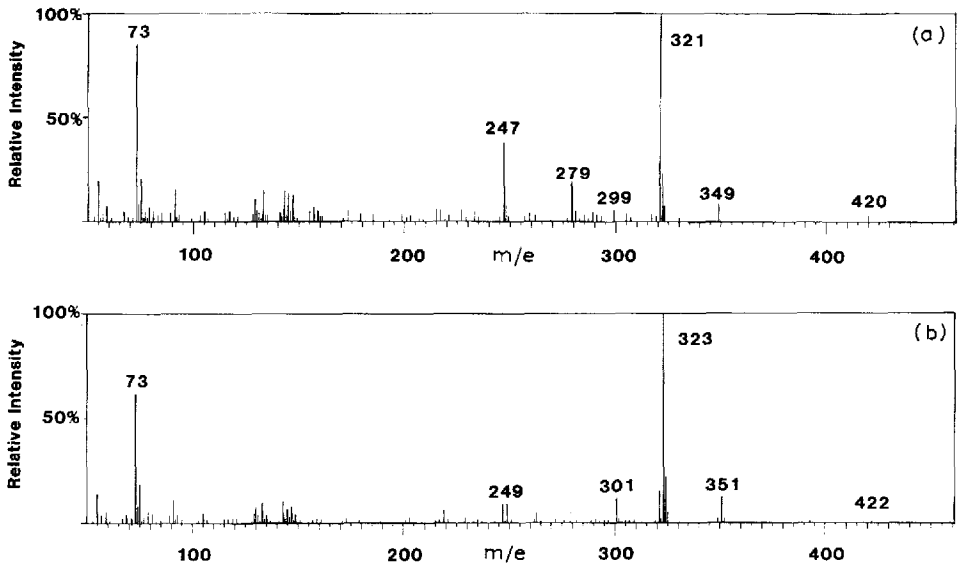

Fig. 5. Mass spectrum of (a) PGB_2 and (b) PGB_1 from frog lung. Purified fractions from the HPLC purification step were methylated with diazomethane and further purified by TLC (diethyl ether–methanol, 98:2). Trimethylsilyl derivatives were prepared using BSTFA (25 μl) and pyridine (10 μl) and analyzed as described in Experimental.

DISCUSSION

The gas chromatographic analysis of the fatty acid composition of *Rana temporaria* lung, heart and urinary bladder tissues showed that in this species, arachidonic acid was the principal 20 carbon fatty acid substrate for prostaglandin synthesis. When the tissues were compared, the heart had the lowest percent of arachidonic acid, while the lung had the highest. The lung also had higher percentages of eicosapentaenoic and eicosatrienoic acids than did the other tissues. The natural abundance for all tissues in decreasing order was arachidonic acid, eicosapentaenoic acid, and eicosatrienoic acid. These results are similar to analysis of these tissues in *Rana pipens*[5,6] and *Rana catesbeiana*[5,10].

Studies with exogenous [1-^{14}C]arachidonic acid suggested that the principal product formed by these frog tissues was a compound with the mobility of PGE_2 on both one and two-dimensional TLC. The inhibition of the radioactive peak in the presence of indomethacin added further support that the product was derived via cyclooxygenase. The conversion of radioactive eicosapentaenoic and eicosatrienoic acids to compounds migrating with PGE_2 suggested PGE compounds may be formed from them as well as arachidonic acid. However, the two-dimensional TLC method used in this study does not discriminate between monoenoic, dienoic, and trienoic prostaglandins.

The development of a method to separate and quantitate PGE_1, PGE_2 and PGE_3 as PGBs allowed evaluation of production of these products by frog tissues. The primary product of *Rana temporaria* lung, heart, and urinary bladder was PGE_2, measured as PGB_2. The structure was verified by mass spectrometry. The largest production of PGE_2 was by urinary bladder. While the studies reported here are for tissue homogenates and would therefore be expected to be considerably higher than baseline values, pilot studies with whole urinary bladder demonstrate that homogenization is not necessary for quantitation of prostaglandins by this method. Values for whole urinary bladder were approximately half those reported here. Heart produced the least PGE_2 of the three homogenized tissues examined. The production of primarily PGE_2 by frog tissues correlates with the fact that arachidonic acid is the most abundant substrate in all three tissues. In additional studies, PGE_2 also appears to be the most abundant E prostaglandin in tissues of *Xenopus laevis*.

Frog lung contained significantly more PGE_1 as verified by MS than did heart or urinary bladder. This may be due to differences in abundance of eicosatrienoic acid in the three tissues. However, eicosatrienoic acid in lung was approximately one-fifth that of arachidonic acid, so the ratio of PGE_1 to PGE_2 is significantly greater than one would predict based on substrate concentration alone. Release of eicosatrienoic acid from membrane phospholipids and/or suitability for the cyclooxygenase may account for its greater than expected conversion to PGE_1. Other reports show a greater production of PGE_1, measured as PGB_1, from exogenous eicosatrienoic acid from urinary bladder than from lung, although the species of *Rana* is not given[11].

The production of PGE_3 in all three tissues was significantly less than that of PGE_1, despite the fact that eicosapentaenoic acid had a greater abundance than eicosatrienoic acid. Eicosapentaenoic acid has been shown to be a poor substrate for mammalian cyclooxygenase and to inhibit arachidonate metabolism[21]. The relative

production of PGE_3 does not appear dependent on relative precursor concentration since the urinary bladder produced the greatest amount of PGE_3 while the lung contained the greatest proportion of its precursor. In this study and others[6,7,9], exogenous eicosapentaenoic acid is efficiently converted into products. This contrast with the low endogenous production of PGE_3 found in the current study. Endogenous release of eicosapentaenoic acid and its subsequent conversion may depend on other regulatory factors not examined in this study.

The tissue content of precursor is clearly not the only factor determining which type of prostaglandins will be synthesized in frogs. As prostaglandins which are structurally related can have different physiological effects in frogs[6,7], the development of this method to quantitate prostaglandins in a species containing several potential precursors is essential to understanding the physiological roles of these compounds in amphibians. In addition, this method can be used to quantitate E prostaglandins in systems which have the potential of utilizing different substrates for prostaglandin production.

ACKNOWLEDGEMENTS

The authors would like to thank Gunter Benthin for his help with the mass spectrometry and Monica D. Lujan for the analysis of fatty acids. The research was supported in part by National Science Foundation Grant No. PCM-8302973 and a Fogarty Senior International Fellowship to C.H. and Swedish Medical Research Council grants 03X-05170 and 03X-05915 to M.H. and E.G.

REFERENCES

1 D. A. van Dorp, R. K. Beerthuis, D. H. Nugteren and H. Vonkeman, *Biochim. Biophys. Acta*, 90 (1964) 204.
2 S. Bergstrom, H. Danielsson and B. Samuelsson, *Biochim. Biophys. Acta*, 90 (1964) 207.
3 M. Hamburg and B. Samuelsson, *J. Biol. Chem.*, 242 (1967) 5336.
4 S. Bergstrom, L. A. Carlson and J. R. Weeks, *Pharmacol. Rev.*, 20 (1968) 1.
5 G. A. Eiceman, V. Fuavao, K. D. Doolittle and C. A. Herman, *J. Chromatogr.*, 236 (1982) 97.
6 C. A. Herman, R. L. Shinholser and M. D. Lujan, *Prostaglandins*, 29 (1985) 629.
7 C. A. Herman, D. V. Gonzales, K. D. Doolittle and L. Jackson, *Prostaglandins*, 21 (1981) 297.
8 C. W. Leffler, R. C. Hanson and E. G. Schneider, *Comp. Biochem. Physiol.*, 66C (1980) 199.
9 C. A. Herman, M. M. McCloskey and K. D. Doolittle, *Gen. Comp. Endocrinol.*, 48 (1982) 491.
10 C. A. Herman, D. O. Robleto, P. L. Mata and M. D. Lujan, *J. Exp. Zool.*, 238 (1986) 167.
11 K. C. Srivastava and T. Mustafa, *Mol. Physiol.*, 5 (1984) 53.
12 E. J. Christ and D. A. van Dorp, *Biochim. Biophys. Acta*, 270 (1972) 537.
13 R. M. Zusman, H. R. Kieser and J. S. Handler, *J. Clin. Invest.*, 60 (1977) 1339.
14 P. Ghiria, L. Parente and D. Piomelli, *Gen. Pharmacol.*, 15 (1984) 309.
15 T. Nomura and H. Ogata, *Biochim. Biophys. Acta*, 431 (1976) 127.
15 R. S. Kent, B. B. Kitchell, D. G. Shand and A. R. Whorton, *Prostaglandins*, 21 (1981) 483.
17 W. S. Powell, *Prostaglandins*, 20 (1980) 947.
18 E. Granström, *Methods Enzymol.*, 86 (1982) 493.
19 M. Hamberg, *Eur. J. Biochem.*, 6 (1968) 135.
20 S. P. Peters, E. S. Schulman, M. C. Liu, E. C. Hayes and L. M. Lichtenstein, *J. Immunol. Methods*, 64 (1983) 335.
21 P. Needleman, M. O. Whitaker, A. Wyche, K. Watters, H. Sprecher and A. Raz, *Prostaglandins*, 19 (1980) 165.