Arachidonate Metabolism in Bovine Gallbladder Mucosa

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Summary: Incubation of [$1\text{-}^{14}\text{C}$] arachidonic acid (AA) with homogenates of bovine gallbladder mucosa in the presence or absence of reduced glutathione (GSH) generated large amounts of products that cochromatographed with PGE_2 and 6-keto- $\text{PGF}_{1\alpha}$ and small amounts of products comigrating with $\text{PGF}_{2\alpha}$, TxB_2 and PGD_2 . In the presence of GSH PGE_2 -like material was predominantly produced while in the absence of GSH 6-keto- $\text{PGF}_{1\alpha}$ -like material was a major product. Identification of PGE_2 was performed by its chemical conversion with NaBH_4 or KOH treatments and by gas chromatography-mass spectrometry. Identification of 6-keto- $\text{PGF}_{1\alpha}$ was confirmed by gas chromatography-mass spectrometry. Gallbladder muscle produced predominantly 6-keto- $\text{PGF}_{1\alpha}$ in the presence or absence of GSH. The total amount of cyclooxygenase products was approximately 2 times higher in the mucosa than in the muscle. These results indicate that bovine gallbladder mucosa contains higher levels of fatty acid cyclooxygenase, and PGE_2 isomerase and PGI_2 synthetase in a proportion different from that in the muscle.

Key words: prostaglandins — arachidonic acid — gallbladder mucosa — gallbladder muscle — glutathione

Introduction

In mammalian gallbladders prostaglandins (PGs) are presumed to play significant roles in fluid transport, mucin secretion and stone formation (Morton et al. 1974; Lee et al. 1981) which are directly associated with mucosal functions. However, PG biosynthesis in the mucosal layers of gallbladders has not been well characterized. Thus, we examined the metabolism of arachidonic acid (AA) in the mucosal layers of bovine gallbladders.

[$1\text{-}^{14}\text{C}$] AA (52 Ci/mol) and [5, 6, 8, 11, 12, 14, 15- ^3H - (N)] PGE_2 (130 Ci/mol) were purchased from New England Nuclear (Boston), AA and indomethacin from Sigma Chemical Co., imidazole and reduced glutathione (GSH) from Tokyo Kasei, and TLC plates of silica gel 60 (0.25 mm, 20 $\times$ 20 cm) from F. Merck (Darmstadt). Authentic PG standards and 15-hydroperoxy-arachidonic acid (15-HPAA) were gifts from Ono Pharmaceutical Company. All other reagents were of the highest grade commercially available.

Materials and Methods

Materials

Preparation of homogenates of mucosal and muscle layers from bovine gallbladders

Bovine gallbladders were obtained from

a local slaughterhouse. The gallbladder was isolated and rinsed with ice-cold saline to remove bile. Mucosal and muscle layers were separated with scissors and tweezers. Serosal and fatty layers were discarded. Tissue sample was homogenized with the same volume of 0.05M Tris-HCl buffer (pH 8.0) containing 0.15M NaCl and 0.5mM dithiothreitol using an Ultra-Turrax homogenizer, and the homogenate was centrifuged at $600 \times g$ for 15 min to remove tissue debris. The supernatant was used as an enzyme preparation.

Assay system for PG biosynthesis

PG biosynthesis was examined as previously described (Nakano et al. 1983). In brief, the homogenate (40 mg protein/ml) was incubated with 20 μ M [$1\text{-}^{14}\text{C}$] arachidonic acid (400,000 dpm) in 10 mM Tris-HCl buffer, pH 8.0, containing 2 mM GSH in a final volume of 2 ml. Reaction was started by adding the substrate dissolved in 20 μ l of ethanol, and continued for 5 min at 37°C with vigorous shaking. The reaction was stopped by acidifying the mixture to pH 3 with formic acid, and radioactive metabolites were extracted with ethyl ether followed by ethyl acetate. The solvent was evaporated under N_2 gas stream, and the dried residue was applied to a TLC plate together with authentic PGE_2 , $\text{PGF}_{2\alpha}$, 6-keto- $\text{PGF}_{1\alpha}$, PGD_2 , PGB_2 and AA. The plate was developed by using the organic phase of the solvent system: isooctane-ethyl acetate-acetic acid-water (25:55:10:50, by volume). Radioactivity on the plate was monitored with an Aloka radiochromatogram scanner model TLC-101 or by autoradiography, and radioactivity of scraped silica gel was counted in an Aloka model LSC 673 liquid scintillation counter. Standard PG spots were visualized with iodine vapor.

Gas chromatography-mass spectrometry (GC-MS)

PGs in the sample were derivatized with diazomethane in ether: methanol (9:1), methoxyamine HCl in pyridine (5 mg/ml) and N, O-trimethylsilyl acetamide (BSA)-trimethyl-chlorosilane (TMCS) before analyses with a JOEL JMS-D100 combined gas chromatograph-mass spectrometer. The 2-meter column (1% OV-1 on Shimalate-W) was kept at 210°C, the flash-heater at 280°C, and the separator at 280°C. Electron energy was set at 30 eV.

Results

A homogenate prepared from mucosa and muscle of gallbladder was incubated with [$1\text{-}^{14}\text{C}$] AA in the presence or absence of GSH. Extract from the reaction mixture was examined by TLC. As shown in Fig. 1A-D, five radiolabeled products (compounds I-V) were formed cochromatographically with 6-keto- $\text{PGF}_{1\alpha}$, $\text{PGF}_{2\alpha}$, TxB_2 , PGE_2 and PGD_2 . Of the total radioactivity recovered from the TLC plates, products I, II, III, IV and V formed in the presence of GSH accounted for 2.1 ± 0.7 , 0.9 ± 0.1 , 0.7 ± 0.1 , 4.9 ± 0.8 and $1.0 \pm 0.6\%$ (total $9.7 \pm 1.9\%$) in mucosal homogenates ($n=6$), and 1.5 ± 0.2 , 0.5 ± 0.1 , 0.3 ± 0.1 , 0.9 ± 0.1 and $1.2 \pm 0.3\%$ (total $4.6 \pm 0.2\%$) in muscle homogenates ($n=4$), respectively. Elimination of GSH from the reaction mixture resulted in a marked decrease of compound IV (to 0.9 ± 0.1) with a concurrent increase of compound I (to 5.6 ± 0.2) in the mucosa ($n=3$), whereas the pattern of products in the muscle ($n=4$) was not affected by omission of GSH.

Fig. 2 shows the time course of the formation of various arachidonate metabolites in the presence of GSH. In the mucosa the major compounds I and IV increased with time for 60 min, and the

Fig. 1. Arachidonic acid transformation by homogenates of bovine gallbladder mucosa or muscle. $[1-^{14}\text{C}]$ AA was incubated with homogenates of gallbladder mucosa (A, B) or muscle (C, D) under standard conditions in the presence (A, C) or absence (B, D) of glutathione. TLC plates were developed twice as described in Materials and Methods. Abbreviation: $6\text{KF}_{1\alpha}$ =6-keto-prostaglandin $\text{F}_{1\alpha}$; $\text{F}_{2\alpha}$, E_2 , D_2 and B_2 =prostaglandin $\text{F}_{2\alpha}$, E_2 , D_2 and B_2 ; TxB_2 =thromboxane B_2 ; org=origin and s.f.=solvent front.

minor compounds II, III and V increased for 20 min reaching a plateau. In the muscle the major product was compound I, which increased depending on the reaction time at least for 60 min.

The amount of the major compounds formed for 5 min increased in proportion to the amount of enzyme protein up to 40 mg. The proportionality was less clear for the minor compounds. A boiled preparation of tissue homogenates produced none of these compounds. Formation of these five products was inhibited by $28\text{ }\mu\text{M}$ indomethacin. Generation of compound I was inhibited by $40\text{ }\mu\text{M}$ 15-HPAA, an inhibitor of PGI_2 synthetase (Gryglewski

et al. 1976), and formation of compound III was prevented by 5 mM imidazole, an inhibitor of Tx synthetase.

For identification of these products chemical conversion and GC-MC analyses were performed. Compound IV was completely converted by NaBH_4 treatment to materials cochromatographing with $\text{PGF}_{2\beta}$ and $\text{PGF}_{2\alpha}$ on TLC in the proportion of 1:1.5. The compound was quantitatively converted by methanolic KOH treatment to a material corresponding to PGB_2 . These chemical conversions were confirmed with authentic $[^3\text{H}]\text{-PGE}_2$ as positive controls. GC-MS of compounds I and IV after derivatization showed mass spec-

Fig. 2. Time courses of AA transformation in gallbladder. Standard reaction mixtures including homogenates of bovine gallbladder mucosa (A) or muscle (B) were incubated in the presence of GSH for the times indicated. Vertical axis indicates conversion rate (mean of 3 experiments) expressed as percentage of the total radioactivity recovered from the TLC plate. ■—■—■: compound I (6-keto-PGF₁α), ▲—▲—▲: compound II (PGF₂α-like material), ×—×—×: compounds III (TxB₂-like material), ●—●—●: compound IV (PGE₂), and ○—○—○: compound V (PGD₂-like material).

tra identical with those of authentic 6-keto-PGF₁α and PGE₂ derivatized in the same way (Fig. 3). The molecular ion and major fragments are in agreement with those reported earlier for the methyl ester-methoxime-trimethylsilyl ether derivative of each corresponding prostaglandin (Gréen, 1969; Chang and Morita, 1977). The amounts of compounds II, III and V were not sufficient for GC-MS.

Discussion

These data indicate that bovine gallbladder mucosa produced predominantly PGE₂ and PGI₂ with lesser amounts of PGF₂α-, TxB₂- and PGD₂-like materials from AA. Furthermore, the predominant product is PGE₂ in the presence of GSH and PGI₂ in its absence.

The requirement of GSH is a specific property of PGE synthetase. Inhibition by 15-HPAA and imidazole is characteristic of PGI₂ synthetase and TXB₂ synthetase, respectively. GSH dependency was not clear in the production of PGD₂-like material. Enzymes involved in the

Fig. 3. Mass spectra of major arachidonate metabolites in gallbladder mucosa. Methyl ester-methoxime-trimethylsilyl ether derivatives of compounds I and IV formed by incubation of 20 μM AA with homogenates of bovine gallbladder mucosa was subjected to GC-MS as described in Materials and Methods. The lower panel shows a spectrum of the major isomer of the derivatized compound IV.

generation of $\text{PGF}_{2\alpha}$ - and PGD-like materials from PGH_2 remain uncertain.

Recent reports (Nakata et al. 1981; Michael et al. 1983) demonstrated that in guinea-pig gallbladders AA was converted predominantly to PGE_2 and to $\text{PGF}_{2\alpha}$, 6-keto- $\text{PGF}_{1\alpha}$ and TxB_2 to a lesser extent. The enzyme preparation used in this study was a homogenate of gallbladders containing morphologically and functionally different layers such as mucosa, muscle and serosa. Inflamed human gallbladders were shown in bioassay to produce PG-like material(s) in different amounts in different layers (Wood and Stanford, 1977). We have now shown that in normal bovine gallbladders the enzymatic capacity to produce PGs in the mucosa is significantly (more than 2 times) higher than in the muscle and that the quantitative pattern of PG production in the mucosa is different from that in the muscle which produces predominantly PGI_2 and to a lesser extent $\text{PGF}_{2\alpha}$ - and PGE_2 -like material (Nakano et al. 1983). GSH availability may control the production of either PGE_2 or PGI_2 as a major product.

These results on the biosynthetic capacity for PGs in the mucosa and muscle should provide a basis for future investigations of the role of PGs in physiological and pathological conditions of the gallbladder.

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