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HIGH-PERFORMANCE LIQUID-CHROMATOGRAPHIC ASSAY FOR PROSTAGLANDINS WITH THE USE OF *p*-(9-ANTHROYLOXY)PHENACYL BROMIDE

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SUMMARY

Gastric mucosa of rats and swine was incubated in buffer for 1 min to produce prostaglandins (PGs). After extraction and derivatization with *p*-(9-anthroyloxy) phenacyl bromide, the prostaglandin esters were determined by high-performance liquid chromatography. A 20-μl sample was injected into a microparticulate silica gel column with a mobile phase of dichloromethane–acetonitrile–methanol (90:9:1). At a flow-rate of 1.5 ml/min the retention times of the prostaglandin esters were 7.14 min (internal standard), 7.90 min (PGF_2), 10.05 min (thromboxane$_2$), 12.26 min (6α-keto-$PGF_{1\alpha}$) and 13.98 min ($PGF_{2\alpha}$). In spite of high sensitivity (0.1 ng per sample) for PGE_2 and $PGF_{2\alpha}$, only PGE_2 synthesis was observed.

INTRODUCTION

Prostaglandins (PGs) are primarily synthesized from arachidonic acid, which is stored in the cell membrane at a concentration normally below 7 μM. Stimuli, such as trauma, infection, and ethanol, perturb phospholipase A_2 activity and cause the release of intracellular arachidonic acid. The increase in free arachidonic acid results in the synthesis of the products of prostaglandin H (PGH) synthase, and the leukotrienes[1]. The oxidation of arachidonic acid via the PGH synthase pathway leads to the formation of PGE_2 $PGF_{2\alpha}$, PGI_2, PGD_2 and thromboxane A_2 (TXA_2). These PGs have been administered to animals and man, and can cause either identical or opposite physiological effects. PGE_2 and PGI_2 are potent exogenous vasodilators and are found in most tissues, TXA_2 is a very powerful vasoconstrictor, $PGF_{2\alpha}$ is a venoconstrictor and a bronchoconstrictor, and PGE_2 is a bronchodilator. Gastric acid secretion is inhibited by PGE_2, but is not affected by $PGF_{2\alpha}$. Although the physiological effect of these PG when administered has been well documented, the role of endogenous prostaglandins in disease and health is still unclear.

Accurate measurement of tissue PG is difficult, and methods which determine the biosynthesis are preferable[2,3]. Recently, we described a high-performance liquid chromatographic (HPLC) assay for PGE_2 generation in gastric mucosa[4]. In this communication the assay for the other products of PGH synthase is described.

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EXPERIMENTAL

Apparatus

A HPLC system (Model 590 delivery module, Model 420 fluorescence detector and Model 730 data module) was purchased from the Waters Chromatography Division of Millipore (Milford, MA, U.S.A.). Samples (20 μl) were injected into a Rheodyne syringe-loading sample injector (Model 7012, Rheodyne, Cotati, CA, U.S.A.) with a 25-μl glass syringe (Hamilton, Reno, NV, U.S.A.). A prepacked microparticulate silica gel column (Zorbax-Sil), Du Pont (Wilmington, DE, U.S.A.), 25 cm × 4.6 mm I.D., was employed at ambient temperature.

Materials

PGE_2, $PGF_{2\alpha}$, 6α-keto $PGF_{1\alpha}$ and TXB_2 were purchased from Upjohn (Kalamazoo, MI, U.S.A.). Panacyl bromide [*p*-(9-anthroyloxy)phenacyl bromide)] was kindly supplied by Dr. Walter Morozowich of Upjohn. Ethyl acetate, acetone, water, dichloromethane, acetonitrile, tetrahydrofuran and methanol were HPLC grade and were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Light petroleum (b.p. 35–60°C) and 88% formic acid were obtained from Mallinckrodt (St. Louis, MO, U.S.A.); triethylamine from J. T. Baker (Phillipsburg, NJ, U.S.A.); Bacto-gelatin from Difco Labs. (Detroit, MI, U.S.A.). All other chemicals were purchased from Sigma (St. Louis, MO, U.S.A.). Silica gel Sep-Pak cartridges were obtained from Waters; high-purity, dry nitrogen was purchased from the T. W. Smith Corp. (Brooklyn, NY, U.S.A.); and polypropylene test tubes from Sarstedt (Princeton, NJ, U.S.A.).

Animals

Holtzman rats, weighing 150–175 g, were purchased from Holtzman (Madison, WI, U.S.A.). Research-breed swine, weighing 25 kg, were purchased from Biomedical Assoc. (Friedensburg, PA, U.S.A.). Animals were fed a standard laboratory chow (Ralston Purina, St. Louis, MO, U.S.A.).

Sample preparation

The procedure for handling tissue and the biosynthesis of PGs has been described previously in detail[5]. In brief, weighed mucosal samples were incubated in a phosphate-buffered saline solution for 1 min. The reaction was terminated, and the PG were extracted by the method of Green *et al.*[6].

13,14-Dihydro-15-keto-$PGF_{2\alpha}$ is a natural oxidation product of $PGF_{2\alpha}$. Since it was not detected in the prostaglandin-generating system used in this study for gastric mucosa, it was used as the internal standard.

13,14-Dihydro-15-keto-$PGF_{2\alpha}$ (50 ng) was added to all samples and standards.

Standards

PGE_2, $PGF_{2\alpha}$, 6α-keto $PGF_{1\alpha}$, TXB_2 and the internal standard were solubilized in acetonitrile at a concentration of 1 mg/ml and stored at −70°C. Dilutions in acetonitrile were made daily for assay. Concentrations were verified by radioimmunoassay. The stable metabolites of PGI_2, 6α-keto $PGF_{1\alpha}$ and of TXA_2, TXB_2, were used to quantitate the amount of metabolite synthesized by the tissue.

Derivative formation

The residue was dissolved in 1 ml acetonitrile–tetrahydrofuran (4:1). PGs (0.04–100 ng) were allowd to react with 10 μg of panacyl bromide in the presence of 1 μl triethylamine for 2 h at room temperature. The reaction mixture was applied to a pre-packed silica gel (Sep-Pak) column, which had been equilibrated with dichloromethane. Excess panacyl bromide was eluted from the column with 20 ml of dichloromethane. PG-panacyl esters were eluted with 2 ml of acetonitrile–methanol (85:15), and the eluent was evaporated under nitrogen. The residue was dissolved in 0.2 ml of acetonitrile.

Chromatographic conditions

The mobile phase consisted of dichloromethane–acetonitrile–methanol (90:9:1). The fluorescence of the PG–panacyl esters was measured at an excitation wavelength of 280 nm with an emission filter having a 400-nm cutoff. Samples or standards (20 μl) were injected into the column for assay, and were determined in duplicate.

RESULTS

When 200 ng of the internal standard, PGE_2, TXB_2, 6α-keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were derivatized, the panacyl esters separated at a flow-rate of 1.5 ml/min as shown in Fig. 1. The internal standard, 13,14-dihydroxy-15-keto-$PGF_{1\alpha}$ and PGE_2 gave peaks at 7.14 and 7.90 min. The other PG had greater base to peak height ratios,

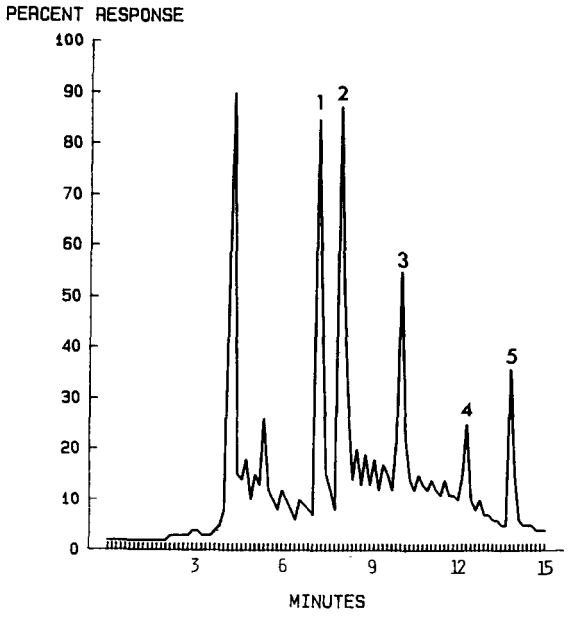

Fig. 1. Chromatogram of the panacyl esters of 13,14-dihydroxy-15-keto-$PGF_{2\alpha}$ (1), PGE_2 (2), TXB_2 (3), 6α-keto-$PGF_{1\alpha}$ (4) and $PGF_{2\alpha}$ (5). The retention times were, 7.14, 7.90, 10.05, 12.26 and 13.98 min, respectively.

and peak heights were less than those of the internal standard and PGE_2. Peak heights of TXB_2 and $PGF_{2\alpha}$ were similar, but that of 6α-keto-$PGF_{1\alpha}$ was less. The retention times of TXB_2, 6α-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ were 10.05, 12.26 and 13.98 min, respectively.

At a flow-rate of 2.3 ml/min, various concentrations of PGE_2–panacyl esters were determined to construct a standard curve. The peak areas varied linearly with the amount injected over a range of 0.04 to 100 ng. With 1 mg PGE_2 the peak area response was 15% below the expected level.

The panacyl esters of several concentrations of $PGF_{2\alpha}$ were also measured. The peak area response against the amount injected was linear from 0.4 to 40 ng. The sensitivity of the assay was ten times less with $PGF_{2\alpha}$. The standard curve for 6α-keto $PGF_{1\alpha}$ was not linear.

Animal studies

In a previous communication, the PGE_2 levels of normal gastric mucosa of rats and swine were reported as 90.6 ± 31.0 and 79.8 ± 39.8 pg/min/mg tissue, respectively[4]. No $PGF_{2\alpha}$, 6α-keto-$PGF_{1\alpha}$, nor TXB_2 biosynthesis was detectable in rat mucosa (150–200 mg) in a 1-min incubation. The lower limit of detection for PGE_2 generation was 2 pg/min/mg tissue, and was 20 pg/min/mg for $PGF_{2\alpha}$ generation.

DISCUSSION

An HPLC assay for PG-panacyl esters has been described in this study, and linear standard curves of peak area response for PGR_2 and $PGF_{2\alpha}$ have been obtained. PGE_2 biosynthesis has been measured in the normal gastric mucosa of rats and swine, but no other product of PGH synthase was detected. Little $PGF_{2\alpha}$ biosynthesis (< 20 pg/min/mg) was not unexpected. While human mucosal homogenates generate PGE_2 and $PGF_{2\alpha}$ in a ratio of 2:1, 6α-keto-$PGF_{1\alpha}$ and TXB_2 occur in only negligible amounts[7]. Other investigators have reported significant amounts of PGI_2 and 6α-keto-$PGF_{1\alpha}$ synthesis in rat mucosa[8]. The extent of mucosal $PGF_{2\alpha}$ synthesis is controversial. Since $PGF_{2\alpha}$ has no effect on gastric acid secretion and a minimal effect on blood flow, minimal endogenous $PGF_{2\alpha}$ generation by the gastric mucosa would be expected.

Although a significant amount of PGI_2 and 6α-keto-$PGF_{1\alpha}$ synthesis by gastric mucosa is likely, no 6α-keto-$PGF_{1\alpha}$ production was detected in our study. The standard curve was not linear, because it is likely that the hemi-ketal intermediate of PGI_2 and the stable end product, 6α-keto-$PGF_{1\alpha}$, reacted with the methanol used in the extraction and mobile phase to form methyl ketals [9]. Thus, the chromatographic conditions described in this study are not suitable for PGI_2 determinations, but are adequate for PGE_2, $PGF_{2\alpha}$ and TXB_2 assays.

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