Fluorometric High Performance Liquid Chromatographic Analysis of Prostaglandin (PG) Metabolites: Application to the Catabolism of PGE₂ and PGF_{2α} in Rat and Rabbit Kidney Homogenates

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A previously reported fluorometric high performance liquid chromatographic procedure for separation of 9-anthryldiazomethane (ADAM) derivatives of prostaglandins (PG's) was applied to examine catabolic products of PG's, such as 15-keto-PGE₂, 13,14-dihydro-15-keto-PGE₂, 15-keto-PGF_{2α}, and 13,14-dihydro-15-keto-PGF_{2α}. ADAM derivatives of these metabolites were separated in 30 min on an ODS column with 65% acetonitrile.

By this method catabolism of PGE₂ and PGF_{2α} in kidney homogenates of rats and rabbits was analyzed. Homogenates of medulla and cortex of rat kidney catabolized PGE₂ and PGF_{2α} to 15-keto-PG's. Whereas the homogenate of rabbit kidney cortex yielded 13,14-dihydro-15-keto-PGE₂, that of medulla did not. The conversion of PGF_{2α} to PGE₂ was shown to occur in homogenates of rabbit kidney cortex as well as rat kidney medulla and cortex.

Keywords — 9-anthryldiazomethane (ADAM); HPLC; rat kidney; rabbit kidney; 15-keto-prostaglandin F_{2α}; 15-keto-prostaglandin E₂; 13,14-dihydro-15-keto-prostaglandin F_{2α}; 13,14-dihydro-15-keto-prostaglandin E₂

Introduction

It is well known that prostaglandins (PG's) in the kidney may have an important role in regulation of renal blood flow and natriuresis.^{1,2)} Since PG's are known to be catabolized rapidly in blood and other body fluids, the rate of renal catabolism of these PG's is a main factor that determines the effect of PG's on renal blood flow and, kidney function. The products of renal catabolism of PG's have been reported;^{3,4)} they have generally been determined by radioimmunoassay (RIA). However, RIA has the problem of radioactive waste, an assay kits are rather expensive.

We previously reported a simple measurement of PG's and thromboxane by high performance liquid chromatography (HPLC) using 9-anthryldiazomethane (ADAM) for derivatization.^{5,6)} In this report we applied the ADAM-HPLC method to the examination of PG catabolism in the kidney.

Materials and Methods

Agents Used — PGF_{2α}, PGE₂, 15-keto-

PGF_{2α} (15-K-PGF_{2α}), 15-keto-PGE₂ (15-K-PGE₂), 13,14-dihydro-15-keto-PGF_{2α} (13,14-DH-15-K-PGF_{2α}) and 13,14-dihydro-15-keto-PGE₂ (13,14-DH-15-K-PGE₂) were gifts from Ono Pharmaceutical Co., Ltd., Osaka. Nicotinamide adenine dinucleotide (NAD⁺) was obtained from Sigma Chemical Co., St. Louis, MO. ADAM was a gift from Funakoshi Pharmaceutical Co., Tokyo.

Preparation of Kidney Homogenates — Male Sprague-Dawley rats (300–500 g, Shizuoka Laboratory Animal Center, Hamamatsu) and male albino rabbits (3–5 kg, Doken Co., Ltd., Ibaragi) were sacrificed by exsanguination. Kidneys were excised, minced, and homogenized with a glass-Teflon homogenizer in 2 volumes (v/w) of potassium phosphate buffer (5 mM) K₂HPO₄, 20% glycerol, pH 7.0). The homogenate was centrifuged at 10000 × *g* for 20 min at 4 °C. The supernatant was collected and kept in ice until used.

Incubation — Supernatant of the kidney homogenate was diluted (10 vol) with phosphate buffer (0.1 M potassium phosphate buffer, pH 7.5), and incubated with PGF_{2α} or PGE₂ (500 ng each) in the presence of NAD⁺ (5 mM)

at 37 °C. The reaction was stopped by addition of sufficient 1 N HCl to bring the final pH to 3.0.

Extraction and ADAM-Derivatization — PG's and their metabolites were extracted as previously reported.^{5,6)} Briefly, the incubation mixtures were extracted through a SEP-PAK C₁₈ (Waters Associates, Milford, MA), and the eluate was evaporated. The residue was dissolved in methanol (50 μ l), mixed with the same volume of 0.2% ADAM solution in ethyl acetate, and left overnight at room temperature. The reaction mixture was evaporated and cleaned by SEP-PAK Silica column chromatography. Finally ADAM derivatives were

eluted with the solvent CH₃CN-CH₃OH (4:1). The eluate was evaporated and the residue was dissolved in CH₃OH:AcOEt (1:1).

Reverse-Phase HPLC (RP-HPLC) — RP-HPLC was performed with a YMC-A303 ODS column (4.6 $\times$ 250 mm; particle size, 5 μ m) at 30 °C with a flow rate of 1.0 ml/min. The mobile phase consisted of CH₃CN/H₂O/H₃PO₄ (65/35/0.1, v/v/v). Pumps (Trirotar V) and injector were from Japan Spectroscopic Co., Tokyo. The fluorescence of the column effluent was continuously monitored using a fluorescence monitor (FP-210, Japan Spectroscopic Co., Tokyo) at 365 (excitation) and 412 (emission) nm.

Results

Figure 1 shows a typical HPLC pattern of ADAM derivatives of authentic PG's, those of PGF_{2 α} , PGE₂, 15-K-PGF_{2 α} , 15-K-PGE₂, 13,14-DH-15-K-PGF_{2 α} , and 13,14-DH-15-K-PGE₂. The above derivatives were eluted and separated in 20–50 min under this isocratic HPLC condi-

Fig. 1. Typical Chromatogram of ADAM Derivatives of Authentic PGF_{2 α} , PGE₂, and Their Metabolites

Authentic PGF_{2 α} , PGE₂, 15-K-PGF_{2 α} , 15-K-PGE₂, 13,14-DH-15-K-PGF_{2 α} , and 13,14-DH-15-K-PGE₂ were derivatized with ADAM, cleaned-up, and injected onto an ODS column. The mobile phase was a solvent consisting of CH₃CN/H₂O/H₃PO₄ (65/35/0.1) at 30 °C, and the flow rate was 1.0 ml/min. Intensity of fluorescence was monitored at 412 nm, with excitation at 365 nm. ADAM derivatives corresponding to the following amounts of the original PG's were injected: 8 ng of PGF_{2 α} and PGE₂, 16 ng of 15-K-PG's, and 32 ng of 13,14-DH-15-K-PG's.

Fig. 2. Calibration Curves of Peak Heights Obtained from HPLC Pattern vs. Original Amount of PGF_{2 α} , PGE₂, and Their Metabolites

Symbols indicate PGF_{2 α} ($\square$), PGE₂ ($\blacksquare$), 13,14-DH-15-K-PGF_{2 α} ($\circ$), 13,14-DH-15-K-PGE₂ ($\bullet$), 15-K-PGF_{2 α} ($\triangle$), 15-K-PGE₂ ($\blacktriangle$).

Fig. 3. Typical HPLC Patterns of PGE₂ Catabolism in Rat Kidney Medulla Homogenate

PGE₂ (500 ng) was incubated with rat kidney medulla homogenate for 5 (A), 15 (B), 40 (C) min in the presence of NAD⁺ (5 mM). The metabolites in the incubation mixture were extracted, derivatized with ADAM, and cleaned-up by SEP-PAK; the concentrated final eluate (representing 10 μ l of original incubation mixture) was injected onto the ODS column.

tion. Figure 2 illustrates calibration curves for these metabolites. Linear regressions were obtained up to 20 ng of each original metabolite. The detection limit was approximately 3 ng under the conditions used.

The method was applied to the detection of the products of PG catabolism in rat kidney. Figure 3 illustrates HPLC patterns indicating the time course of catabolism of PGE₂ in a rat kidney medulla homogenate. The peak of PGE₂ decreased 25% by the end of the initial 5 min, 47% by 15 min and 80% by 40 min. Peaks corre-

sponding to 15-K-PGE₂ and 13,14-DH-15-K-PGE₂ increased with time.

By this HPLC method, comparison of catabolic products was made between rat and rabbit kidney homogenates. Figure 4 shows the catabolism of PGF_{2 α} in rat and rabbit kidney homogenates. Panels A and B indicate metabolism of PGF_{2 α} in rat kidney medulla and cortex, respectively, while panels C and D shows metabolism of PGF_{2 α} in rabbit kidney medulla and cortex, respectively. With rat kidney medulla homogenate PGF_{2 α} was metabolized mainly into the following 4 products within 40 min, in the order indicated: 13,14-DH-15-K-PGE₂ > 15-K-PGE₂ > PGE₂ > 15-K-PGF_{2 α} . With cortex homogenate, the order was 15-K-PGE₂ > 13,14-DH-15-K-PGE₂, PGE₂, 15-K-PGF_{2 α} , even though the decrease in PGF_{2 α} level was similar to that in the medullary homogenate and PGE₂ was rapidly produced during the initial 5–10 min in both homogenates. Rabbit kidney medulla, only small amounts of metabolites were found under the same conditions. With cortex, 4 products, 13,14-DH-15-K-PGE₂, 15-K-PGF_{2 α} , PGE₂, and 13,14-DH-15-K-PGF_{2 α} , were found.

Regarding the catabolism of PGE₂, this PG was converted in the rat kidney medulla homogenate into 15-K-PGE₂ and the main product, 13,14-DH-15-K-PGE₂, whereas in the cortex homogenate the main product was 15-K-PGE₂, with only a small amount of 13,14-DH-15-K-PGE₂. Rabbit kidney medulla was less active than cortex, which yielded 13,14-DH-15-K-PGE₂ and 15-K-PGE₂ (Fig. 5).

Discussion

The ADAM-HPLC method was applied to the examination of catabolic products of PGF_{2 α} or PGE₂ formed by kidney homogenate. The detection limit of each metabolite was approximately 3 ng. The sensitivity of this method was sufficient for assessment of PG catabolism by a kidney homogenate (about 1 mg protein) in a incubation mixture containing 500 ng of PGE₂ or PGF_{2 α} . No significant interfering peak was found in the chromatogram around the peaks of the metabolite produced. Thus, this method can be used widely for examination of PG's and

Fig. 4. Time Courses of Catabolism of $\text{PGF}_{2\alpha}$ by Rat or Rabbit Kidney Homogenates

A) rat kidney medulla, B) rat kidney cortex, C) rabbit kidney medulla, D) rabbit kidney cortex. Each homogenate (approximately 1 mg protein in a 500- μl incubation mixture) was incubated with $\text{PGF}_{2\alpha}$ (500 ng) in the presence of NAD^+ (5 mM). $\text{PGF}_{2\alpha}$ and its metabolites in the incubation mixture were extracted by a SEP-PAK C_{18} . The extracts were derivatized with ADAM, cleaned-up, and loaded onto the HPLC column. Symbols indicate $\text{PGF}_{2\alpha}$ ($\square$), 15-K- $\text{PGF}_{2\alpha}$ ($\triangle$), 13,14-DH-15-K- $\text{PGF}_{2\alpha}$ ($\circ$), PGE_2 ($\blacksquare$), 15-K- PGE_2 ($\blacktriangle$) and 13,14-DH-15-K- PGE_2 ($\bullet$). The amount of each catabolite was determined from the calibration curves (Fig. 2). Each point, which is calculated as percent of the amount of substrate, represents the mean $\pm$ S.E. ($n = 3$).

their catabolism in various tissues.

By using this method we compared catabolism of $\text{PGF}_{2\alpha}$ and PGE_2 in rat and rabbit kidney homogenates. The results indicate that rat kidney, both medulla and cortex, can rapidly convert $\text{PGF}_{2\alpha}$ to PGE_2 and the latter into the 15-keto product and then further to the 13,14-dihydro product.

Rabbit kidney has less activity for this conversion; only the cortex has weak converting activity. Regarding catabolism of PGE_2 , rat kidney also showed high activity. Rabbit kidney also catabolized PGE_2 only in cortex homogenate.

These results agree mostly with previous results, which demonstrated that the main site of renal PG catabolism is the cortex in rabbit³⁾ or swine⁷⁾ kidney, but both cortex and medulla in rat.⁸⁾ We detected smaller amounts of 15-K-metabolites among the metabolites in the rabbit kidney homogenate than those of rat. One reason for this could possibly be that activity of PG- Δ^{13} -reductase in rabbit kidney is rather high so that 15-K-metabolites generated are immediately catabolized to 13,14-DH-15-K-metabolites, especially in the case of PGE_2 catabolism.

Fig. 5. Time Courses of Catabolism of PGE₂ by Rat or Rabbit Kidney Homogenates

A) rat kidney medulla, B) rat kidney cortex, C) rabbit kidney medulla, D) rabbit kidney cortex. Each homogenate (same amount of each as in Fig. 4) was incubated with PGE₂ (500 ng) in the presence of NAD⁺ (5 mM). PGE₂ and its metabolites in the incubation mixture were derivatized with ADAM and assayed by HPLC. Symbols indicate PGE₂ (■), 15-K-PGE₂ (▲), and 13,14-DH-15-K-PGE₂ (●). The amount of each metabolite was determined from the calibration curves shown in Fig. 2. Each point, which is calculated as percent of the amount of the substrate, represent the mean $\pm$ S.E. ($n=3$).

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