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Short Communication

Liquid chromatography and radioimmunoassay method for the determination of prostaglandins E_1 and E_2 in rat embryo incubates

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ABSTRACT

This paper describes the application of a combined high-performance liquid chromatography and radioimmunological assay method for the measurement of prostaglandins E_1(PGE_1) and E_2(PGE_2). Samples were acidified to pH 3.15, extracted twice with ethyl acetate and further processed through C_{18} solid-phase extraction cartridges. After HPLC purification, PGE_1 and PGE_2 were measured by radioimmunological techniques. The limit of detection for PGE_1 was 3.9 pg/ml and the intra-assay relative standard deviation was 7.8% for $n = 5$. The accuracy of the assay procedure was also verified. The method has been applied to the determination of PGE_1 and PGE_2 in embryo incubates from 10-day pregnant rats.

INTRODUCTION

Prostaglandin E_1(PGE_1) is a physiologically important metabolite of dihomo-γ-linolenic acid whose concentrations in human tissues are approximately one quarter of those of arachidonic acid (AA) [1]. PGE_1 has been virtually ignored in the HPLC–radioimmunoassay (RIA) profiling studies reported to date. The reason for such an omission could be that, in practice, PGE_1 is a minor metabolite compared with other AA cyclooxygenase metabolites such as prostaglandin E_2(PGE_2), although relatively elevated concentrations of up to 16 μg/ml PGE_1 have been determined in human seminal plasma [2–3].

In general, papers dealing with the HPLC separation of PGE_1 from other prostanoids are scarce [4], especially when these HPLC methods have to be combined with RIA. Taking this into account, we have evaluated an HPLC–RIA method for concurrent determination of PGE_1 and PGE_2. This method has been applied to a study of the release of both prostaglandins by 10-day old rat embryos.

EXPERIMENTAL

Rat embryo incubations

Pools of three embryos obtained from rats

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after 10 days of pregnancy plus the covering membranes and free of maternal decidua were incubated in Krebs–Ringer–hydrogencarbonate (KBR) solution containing 11.0 m*M* glucose, in a metabolic shaking bath under an atmosphere of 5% carbon dioxide in 95% oxygen [5]. After 60 min of incubation, the incubating medium was recovered and acidified to pH 3.15 with 1 *M* hydrochloric acid. Finally, the prostaglandins were extracted three times with 2 ml of ethyl acetate and subsequently evaporated to dryness under a helium stream. Samples were stored at −80°C until assay.

Analyses of prostanoids

C_{18} Solid phase extraction. Dried ethyl acetate residues were resuspended in saline and acidified to pH 3.15 with 1 *M* hydrochloric acid. Following this, the samples were processed through C_{18} solid-phase extraction cartridges, as previously described [6]. Briefly, the cartridges were washed with 10 ml of acidified water (pH 3.15) and 20 ml of light petroleum ether. The prostaglandins were finally eluted with 4.5 ml of methyl formate, which was evaporated to dryness. Dried residues were resuspended in a formic acid/0.04 *M* triethylamine buffer–acetonitrile mixture (65:35) at pH 3.15 and 200-μl aliquots of this solution were analysed by HPLC.

High-performance liquid chromatography. Reversed phase-HPLC separation was performed using two Kontron 414 pumps connected to a Rheodyne 7125 injector and an ODS-2 column (300 × 3.9 mm I.D.), which was isocratically eluted with formic acid/0.04 *M* triethylamine–acetonitrile (65:35 at pH 3.15), as previously described [7]. Samples were collected at the retention times previously established for authentic standards of tritiated PGE_1(53.7 Ci/mmol) and PGE_2(184 Ci/mmol) by a Ray Test Ramona (Issomess, Strabenhardt, Germany) radioactivity detector coupled to the HPLC column. Collected eluates were lyophilized and redissolved in 100 m*M* Tris–HCl buffer pH 7.4 for subsequent radioimmunological assay.

Radioimmunological assay. PGE_1 and PGE_2 were measured in duplicate using specific rabbit antisera from the Pasteur Institute (Marnes La Coquete, Paris, France). Cross-reactivities of PGE_1 antiserum with other prostaglandins were as follows: 6-keto-$PGF_{1\alpha}$, 0.010%; thromboxane B_2, 0.010%; PGE_2, 15%; and PGD_2, 0.10%. Immunoassays for PGE_1 were peformed with tritiated PGE_1 as radioligand (24 pg, 40.7 Ci/mmol) and the above-mentioned antiserum at a final dilution of 1:44. A standard curve was prepared with authentic PGE_1 from 1.9 to 500 pg (nine points) diluted in 100 m*M* Tris–HCl buffer pH 7.4. HPLC eluates were taken up in 250 μl of buffer and the assays were carried out with 100-μl aliquots. In all cases, the samples and standards were incubated with a mixture of antisera and known tritiated standard at 4°C for 24 h. After incubation, prostaglandins were adsorbed in dextran-coated charcoal and the tritium remaining in supernatants was counted on an LKB1217 Racbeta (LKB, Turku, Finland). The unknowns were compared with a standard curve in which the logarithm of the concentration was plotted against the logit of B/B_0 values [the proportion of tracer bound (B) expressed as a percentage of tracer in the zero standard (B_0)]. Cross-reactivities of PGE_2 antiserum were 15% for PGE_1 and lesser than 0.01% for TXB_2, 6-keto $PGF_{1\alpha}$ and PGD_2. The radioimmunoassay for PGE_2 was performed as previously described [5,6].

RESULTS AND DISCUSSION

Fig. 1 shows the complete separation of tritiated 6-keto prostaglandin $F_{1\alpha}$, thromboxane B_2, prostaglandin E_2 and prostaglandin E_1 accomplished within 16 min. Under these experimental conditions prostaglandin D_2 practically co-elutes with PGE_1 (data not shown) [8]. The high volatility of the mixture of formic acid/0.04 *M* triethylamine–acetonitrile (65:35 at pH 3.15) facilitates the lyophilization and subsequent radioimmunological assay of both prostaglandins in the same HPLC fraction [8]. This is made possible by the high specificity of the PGE_1 antiserum as its cross-reactivity with PGD_2 is less than 0.10%. This HPLC separation has been routinely employed in our laboratory for simultaneous HPLC–RIA determination of series 2 prostanoids in human urine [9]. The method herein described could also be very useful for the

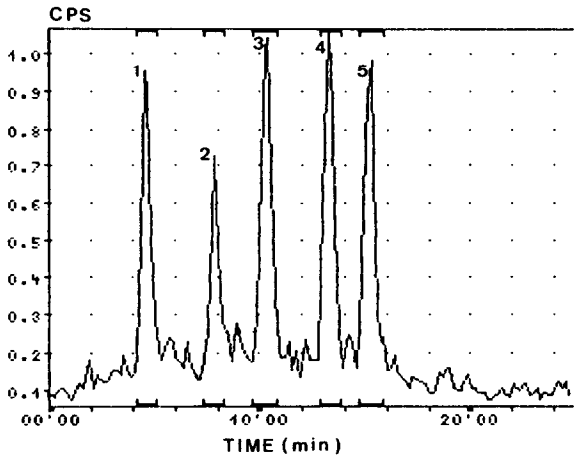

Fig. 1. Typical radiochromatography profile obtained with standards of tritiated 6-keto prostaglandin $F_{1\alpha}$ (1), thromboxane B_2 (2), prostaglandin $F_{2\alpha}$ (3), prostaglandin E_2 (4) and prostaglandin E_1 (5). The HPLC column was an ODS-2, 30 cm × 3.9 mm I.D. column eluted isocratically with formic acid/0.04 *M* triethylamine buffer–acetonitrile (65:35) pH 3.15. Flow-rate was 1 ml/min. Heavy bars in the abcissa define the collection time window set for this study.

concurrent analysis of urinary PGE_1 and PGE_2, especially in biomedical studies in which seminal contamination must be discounted [10]. It is known that PGE_1 and PGE_2 are major components of seminal plasma [11].

Overall recoveries obtained for tritiated PGE_1 and PGE_2 after ethyl acetate, C_{18} solid phase extractions and HPLC purification were higher than 65% for each prostaglandin.

The limit of detection of the assay for PGE_1 was *ca.* 3.9 pg/ml, as determined by the amount of standard displacing 10% of the bound radioactivity and by the mean plus two standard deviations of the ten zero-standards. The intra-assay variation coefficient established for five consecutive measurements was 7.8%. Biological samples in this study were assayed within the same analysis.

To test the accuracy of the procedure, HPLC aliquots of PGE_1 eluates collected from extracted incubates were enriched with known amounts of PGE_1 (3.9–125 pg). As shown in Fig. 2, a close linear correlation was established between the added and measured amounts of PGE_1 ($r = 0.9974$, slope = 0.978). Also, as indicated in Table I, the parallelism test using

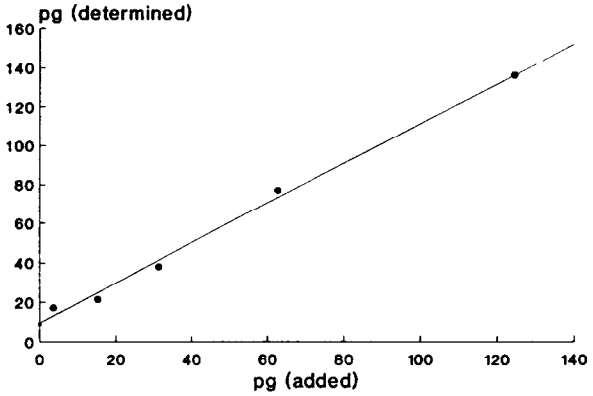

Fig. 2. Correlation plots of RIA PGE_1 values obtained from HPLC aliquots of extracted and supplemented incubate pools.

serially diluted aliquots of biological samples subjected to the whole procedure (liquid–liquid extraction, C_{18} solid-phase extraction, HPLC and RIA) gave good correlations for both series of samples ($r = 0.9953$, slope = 0.973).

The method has been applied to the determination of PGE_2 and PGE_1 levels in rat incubates, as shown in Fig. 3. These data reveal that the PGE_2 production in rat embryos is about four times higher than that of PGE_1, and also confirm the suitability of the assay when both prostaglandins must be measured by liquid chromatography combined with radioimmunoassay.

In conclusion, the HPLC–RIA techniques described herein; provide a specific, sensitive

TABLE I

SERIAL DILUTION OF A POOL OF EMBRYO INCUBATES SUBJECTED TO RIA. RESULTS FOR PGE_1 VALUES EXPRESSED AS pg/ml

Regression line: $n = 5$, $r = 0.9953$, slope = 0.973.

Dilution	Blank incubates	
	Measured	Expected
1:1	291.3	–
1:2	147.1	145.6
1:4	85.9	72.8
1:8	42.4	36.4
1:16	18.8	18.2
1:32	8.6	9.1

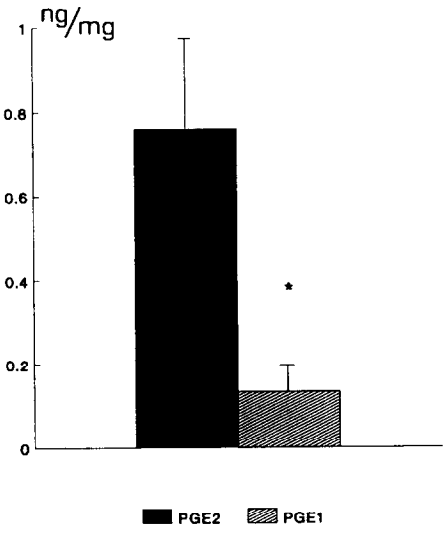

Fig. 3. PGE_1 and PGE_2 levels (ng per mg of protein) in rate embryo incubates ($n = 5$). $^*P < 0.05$ (Student's t-test).

and accurate method for PGE_1 assay in biological samples. The method could be useful for studying the relevance of PGE_2 and PGE_1 in relation to alterations of glucose metabolism in embryo and uterus from diabetic rats.

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