RADIOIMMUNOASSAY OF PROSTAGLANDINS E_2 AND $F_{2\alpha}$ IN CELL CULTURE MEDIA UTILIZING ANTISERA AGAINST PROSTAGLANDINS $F_{2\beta}$ AND $F_{2\alpha}$

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1. Introduction

Early determinations of prostaglandins were based on chromatographic separations followed by bioassays [1–3]. More recently, methods such as fluorimetry of NADH generated by prostaglandin dehydrogenase [4], gas-liquid chromatography with electron capture detector [5,6], and quantitative mass spectrometry using deuterium labeled prostaglandins as internal standards [7] have been developed. Several radioimmunoassays for prostaglandins have also been described [8–16].

Although there are reports on radioimmunoassays for prostaglandin E_2 (PGE_2)*, several authors have reported difficulties in producing antisera against E-type prostaglandins [8,11,12,14] probably due to dehydration of PGE's under the conditions used to attach the prostaglandin to a protein. We therefore developed a method to measure PGE_2 and $PGF_{2\alpha}$ in cell culture media which is based on $NaBH_4$ reduction of the samples followed by radioimmunoassay of $PGF_{2\alpha}$ and $PGF_{2\beta}$. An evaluation of the method by quantitative mass spectrometry is also presented.

* Abbreviations: PGA_2: 15(S)-hydroxy-9-ketoprosta-5-*cis*, 10,13-*trans*-trienoic acid; PGB_2: 15 (S)-hydroxy-9-ketoprosta-5-*cis*, 8(12),13-*trans*-trienoic acid; PGE_1: 11α,15(S)-dihydroxy-9-ketoprost-13-*trans*-enoic acid; PGE_2: 11α, 15 (S)-dihydroxy-9-ketoprosta-5-*cis*,13-*trans*-dienoic acid; $PGF_{1\alpha}$: 9α,11α,15(S)-trihydroxyprost-13-*trans*-enoic acid; $PGF_{2\alpha}$:9α, 11α,15 S-trihydroxyprosta-5-*cis*, 13-*trans*-dienoic acid; $PGF_{2\beta}$: $9_\beta 11_\alpha$, 15 (S)-trihydroxyprosta-5-*cis*, 13-*trans*-dienoic acid; 13,14-dihydro-15-keto PGE_2: 11α-hydroxy-9,15-diketoprosta-5-*cis*, 13-*trans*-dienoic acid; 13,14-dihydro $PGF_{2\alpha}$: 9α,11α, 15(S)-trihydroxyprost-5-*cis*-enoic acid; 13,14-dihydro-15-keto $PGF_{2\alpha}$: 9α,11α-dihydroxy-15-ketoprost-5-*cis*-cnoic acid; 15-keto $PGF_{2\alpha}$: 9α,11α-dihydroxy-15-ketoprosta-5-*cis*, 13-*trans*-dienoic acid.

2. Materials and methods

2.1. *Antisera*

An antiserum against $PGF_{2\beta}$ was obtained from rabbits after immunization with a [9α-^{3}H] $PGF_{2\beta}$-bovine serum albumin conjugate as described earlier [15]. The conjugate was prepared with *N,N'*-carbonyldiimidazole as coupling reagent [17]. $PGF_{2\alpha}$ antiserum was a generous gift of Dr K. T. Kirton of the Upjohn Company, Kalamazoo, Michigan. The antisera were diluted 1:400 v/v with 0.05 M Tris–HCl, pH 8.0 (final dilution: 1:2800).

2.2. *Unlabeled prostaglandins*

Prostaglandins A_2, B_2, E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, 15-keto PGE_2, 13,14-dihydro-15-keto PGE_2, 15-keto $PGF_{2\alpha}$, 13,14-dihydro-15-keto $PGF_{2\alpha}$ and 13,14-dihydro $PGF_{2\alpha}$ were kindly given by Dr J. E. Pike of the Upjohn Company. Prostaglandin $F_{2\beta}$ was prepared from PGE_2 as described before [18]. Standard solutions of prostaglandins were prepared in 0.05 M Tris–HCl, pH 8.0 or redistilled water.

2.3. *Tritium labelled prostaglandins*

[9α-^{3}H]$PGF_{2\beta}$ (specific activity: 0.2 mCi/mmole), [17,18-$^3H_2$] $PGF_{2\alpha}$ and [17,18-$^3H_2$] $PGF_{2\beta}$ (specific activities: 22.5 Ci/mmole) were prepared as described previously [19,20]. The radioactive prostaglandins were dissolved in 0.05 M Tris–HCl, pH 8.0.

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2.4. *$NaBH_4$ reductions and radioimmunoassay*

A 0°C solution of 5 mg $NaBH_4$/ml of water was prepared immediately before use.

For each experiment three series of test tubes were placed on ice. To two of the series 0.1 ml of $NaBH_4$ solution and to the third 0.2 ml of 0.05 M Tris–HCl, pH 8.0, were added per tube. Aliquots (0.1 ml) of cell culture media to be analyzed were added to three tubes in each series. To the remaining tubes was added 0.1 ml of standard solution: $PGF_{2\beta}$ standards to one series containing $NaBH_4$ and $PGF_{2\alpha}$ standards to the two other series of test tubes. The standard solutions contained increasing amounts of prostaglandins from 25 to 3200 pg/0.1 ml. The tubes containing $NaBH_4$ were maintained at room temperature for 10 min and then the excess $NaBH_4$ was destroyed by adding 0.1 ml of 0.1 M HCl. Finally 0.2 ml of 0.05 M Tris–HCl, pH 8.0 containing 5 mg/ml of bovine gammaglobulin (Fraction II; Miles Laboratories Inc. Kankakee, Illinois) was added to each of the tubes in the three series, and the mixtures were left overnight at 4°C. After the tubes had reattained room temperature 0.1 ml of diluted antiserum was added ($PGF_{2\beta}$ antiserum to the $NaBH_4$ treated series with $PGF_{2\beta}$ standards; $PGF_{2\alpha}$ antiserum to the remaining tubes). After 30 min, 0.1 ml of [17,18-$^3H_2$]$PGF_{2\beta}$ (ca 50 pg; 7000 dpm) was added to the tubes containing $PGF_{2\beta}$ antiserum and 0.1 ml of [17,18-$^3H_2$]$PGF_{2\alpha}$ (approx. 50 pg; 7000 dpm) to the tubes containing $PGF_{2\alpha}$ antiserum. The mixtures were kept at room temperature for 45 min and at 0°C for 30 min. Cold 25% (w/v) aqueous polyethylene glycol 4000 (Kebo AB, Stockholm, Sweden) was then added (0.7 ml/tube) [13]. The contents were mixed until a uniform cloudiness appeared and then centrifuged at 1400 *g* and 4°C for 45 min. One ml of the clear supernatants were transferred to Packard vials and counted in 10 ml of modified Bray's scintillation solution [13] using a Packard Tri Carb model 3385 liquid scintillation spectrometer, equipped with automatic external standardization. Calculations were performed off-line on a Digital Equipment's PDP8I computer.

3. Results and discussion

An antiserum against $PGF_{2\beta}$ was obtained by immunizing rabbits with a [9α-^{3}H]$PGF_{2\beta}$-bovine serum albumin conjugate. A dissociation constant of 0.2 nM and a concentration of binding sites of 62 pmoles/mg of protein were found from Scatchard plots [21] for the interaction between $PGF_{2\beta}$ and the antiserum. Specificity studies were also carried out and the results are shown in table 1. The dissociation constant for the interaction between $PGF_{2\alpha}$ and its antiserum was 0.9 nM and the concentration of binding sites 45 pmoles/mg of protein. Table 2 shows the results of our specificity studies for this antiserum.

Table 1
Specificity of the $PGF_{2\beta}$ antiserum

Prostaglandin	Picograms required to displace 50% of bound tracer	Relative cross reaction %
Untreated		
$PGF_{2\beta}$	650	100
$PGF_{1\alpha}$	23 000	2.8
$PGF_{2\alpha}$	11 600	5.6
PGE_1	5 200	12.5
PGE_2	3 000	22.0
PGA_2	72 000	0.9
PGB_2	> 650 000	< 0.1
13,14-dihydro $PGF_{2\alpha}$	46 000	1.4
15-keto $PGF_{2\alpha}$	160 000	0.4
13,14-dihydro-15-keto $PGF_{2\alpha}$	> 650 000	< 0.1
15-keto PGE_2	> 650 000	< 0.1
13,14-dihydro-15-keto PGE_2	> 650 000	< 0.1
$NaBH_4$-treated		
$PGF_{2\beta}$	550	100
$PGF_{1\alpha}$	13 750	4.0
$PGF_{2\alpha}$	7 400	7.4
PGE_1	1 500	36.7
PGE_2	860	64.0
PGA_2	18 000	3.1
PGB_2	> 550 000	< 0.1
13,14-dihydro $PGF_{2\alpha}$	34 000	1.6
15-keto $PGF_{2\alpha}$	9 300	5.9
13,14-dihydro-15-keto $PGF_{2\alpha}$	50 000	1.1
15-keto PGE_2	5 500	10.0
13,14-dihydro-15-keto PGE_2	69 000	0.8

Initial experiments showed that at a concentration of 5 mg/ml, $NaBH_4$ completely reduced at least 4 μg of ^{3}H labeled PGE_2/ml of cell culture medium (room temperature, 10 min) to a mixture of $PGF_{2\alpha}$ and $PGF_{2\beta}$. In the ensuing radioimmunoassays we used polyethylene glycol precipitation to separate antibody-bound and free $PGF_{2\alpha}$.

Table 2
Specificity of the $PGF_{2\alpha}$ antiserum

Prostaglandin	Picograms required to displace 50% of bound tracer	Relative cross reaction %
Untreated		
$PGF_{2\alpha}$	800	100
$PGF_{1\alpha}$	1 650	48.4
$PGF_{2\beta}$	53 000	1.5
PGE_1	> 800 000	< 0.1
PGE_2	> 800 000	< 0.1
PGA_2	> 800 000	< 0.1
PGB_2	> 800 000	< 0.1
13,14-dihydro $PGF_{2\alpha}$	13 800	5.8
15-keto $PGF_{2\alpha}$	28 000	2.8
13,14-dihydro-15-keto $PGF_{2\alpha}$	160 000	0.5
15-keto PGE_2	> 800 000	< 0.1
13,14-dihydro-15-keto PGE_2	> 800 000	< 0.1
$NaBH_4$-treated		
$PGF_{2\alpha}$	725	100
$PGF_{1\alpha}$	1 475	49.2
$PGF_{2\beta}$	36 000	2.0
PGE_1	4 300	16.9
PGE_2	2 200	33.0
PGA_2	43 000	1.7
PGB_2	> 725 000	< 0.1
13,14-dihydro $PGF_{2\alpha}$	12 000	6.0
15-keto $PGF_{2\alpha}$	1 050	69.1
13,14-dihydro-15-keto $PGF_{2\alpha}$	14 500	5.0
15-keto PGE_2	13 500	5.4
13,14-dihydro-15-keto PGE_2	130 000	0.6

To measure PGE_2 and $PGF_{2\alpha}$ concentrations in cell culture media three radioimmunoassays were carried out: $PGF_{2\beta}$ concentrations were measured after $NaBH_4$ reduction and $PGF_{2\alpha}$ concentrations were measured both before and after reduction. Since there was some cross reaction between $PGF_{2\alpha}$ and the $PGF_{2\beta}$ antiserum (table 1) the $PGF_{2\beta}$ values had to be corrected. PGE_2 was determined as the sum of $[PGF_{2\alpha}]$ after reduction$-[PGF_{2\alpha}]$ before reduction and the corrected $[PGF_{2\beta}]$.

To test the radioimmunoassay, known amounts of prostaglandins E_2 and $F_{2\alpha}$ were added to fresh Eagle's minimum essential medium (Gibco) supplemented with 10% fetal bovine serum (Biocult). Aliquots of the media were analyzed in triplicates as described in section 2.4., and the entire analysis was performed three

Table 3
Radioimmunoassay of known amounts of PGE_2 and $PGF_{2\alpha}$ added to culture medium

Medium		Amount added, ng/ml	Medium level found ng/ml
I	PGE_2	0.00	0.54 ± 0.33*
	$PGF_{2\alpha}$	0.00	1.32 ± 0.62
II	PGE_2	3.00	2.53 ± 0.29
	$PGF_{2\alpha}$	1.50	2.87 ± 0.40
III	PGE_2	6.00	5.81 ± 0.20
	$PGF_{2\alpha}$	2.00	3.38 ± 0.27
IV	PGE_2	12.00	11.01 ± 0.74
	$PGF_{2\alpha}$	6.00	7.79 ± 0.22
V	PGE_2	4.00	3.76 ± 0.31
	$PGF_{2\alpha}$	4.00	5.05 ± 0.31
VI	PGE_2	8.00	7.70 ± 0.37
	$PGF_{2\alpha}$	3.00	4.36 ± 0.38

Different amounts of PGE_2 and $PGF_{2\alpha}$ in 10 µl EtOH were added to 25.0 ml of fresh medium. The assay was carried out as described in the text.
* S.D.: 3 separate RIA determinations.

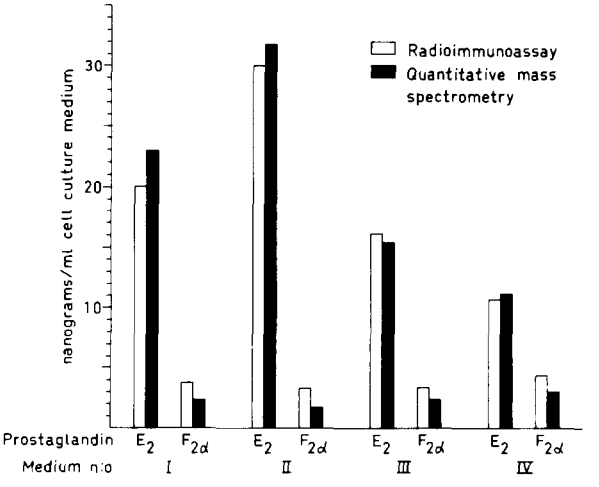

Fig. 1. Comparison between quantitative mass spectrometry and radioimmunoassay of prostaglandins E_2 and $F_{2\alpha}$ in growth media obtained from four different cultures of 3T3 cells. The radioimmunoassay was carried out as described in the text.

times on separate occasions. Table 3 shows the results from the three analyses expressed as mean values ± standard deviations. The values obtained for media with no added prostaglandins were due to endogenous prostaglandins in the calf serum.

For further evaluation of the method, growth media from cultures of 3T3 cells (Dulbecco's modified Eagle's medium supplemented with 10% calf serum, both from Gibco) were analyzed both by radioimmunoassay and by quantitative mass spectrometry as described before [22]. The results obtained by the two methods were in good agreement as is shown in fig. 1. The present radioimmunoassay should therefore be a useful procedure for the rapid determination of PGE_2 and $PGF_{2\alpha}$ concentrations in large numbers of cell culture media.

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