VALIDITY OF PG E_1 RADIOIMMUNOASSAY BY USING PG E_1 ANTISERA WITH DIFFERENTIAL BINDING PARAMETERS

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1. Introduction

Prostaglandins (PGs) become immunogenic when their carboxyl groups are coupled by carbodiimide to free NH_2 groups of proteins. Thus it is possible to raise PG Fα antibodies directed mainly against PG Fα [1–3]. But this procedure, when used for the other types of primary prostaglandins, has rarely been successful for production of antibodies with specificity for the homologous hapten. Antibodies obtained in rabbits by immunizing with prostaglandin E_1-bovine serum albumin (PG E_1-BSA) were directed mainly against PG A_1 [4] or PG B_1 [5,6] or did not discriminate between PG A_1 and PG B_1 [7]; antibodies to PG E_2-BSA were directed against more PG A_2 and PG B_2 than PG E_2 [8,9]. Antibodies to PG A_1-BSA cross reacted significantly against PG E_1 and PG E_2 [4] or to a higher extent against PG B_1 [7]; and antibodies to PG B_2-BSA cross reacted to a higher extent with PG B_1 (unpublished data).

Despite the poor specificity of these antibodies, a certain credence has been given to PG E_1 and PG A_1 radioimmunoassays [10] when appropriate chromatography is introduced into the procedure.

This report describes the binding parameters of antibodies raised in rabbits and sheep by immunization with the same immunogen PG E_1-BSA and presents a comparative study of PG E_1 levels in human plasma using the rabbit and sheep antisera of the greatest specificity. The effects of greater affinity and specificity of the sheep antisera will be discussed.

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2. Materials and methods

Prostaglandins were a generous gift of Dr John E. Pike, Upjohn Co. Tritiated PG E_1 (110 Ci/mM) was purchased from New England Nuclear and its chemical purity regularly checked with silicic acid chromatography before use [4].

The immunogen was prepared by the coupling of PG E_1 to bovine serum albumin (BSA) with carbodiimide as previously described [6]. Five rabbits were primed with 1 mg of the immunogen in complete Freund's adjuvant and boosted according to the schedule previously described [6].

The same immunogen (3 mg PG E_1-BSA) emulsified in complete Freund's adjuvant was used to prime one sheep; booster injections (2 mg PG E_1-BSA) were given intravenously every two months. Blood was collected weekly beginning two months after priming. Each bleeding was examined for its titre, sensitivity and specificity. Average affinity constants, $K_{a(av)}$, were determined using dextran coated charcoal (Norit A, 250 mg, Dextran T 70, 25 mg/100 ml of gelatin phosphate buffer saline) to separate bound from free PG E_1 [11]; calculations were done according to Scatchard [12]. Blood samples from healthy subjects were collected in vacutainers containing dry EDTA (14 mg) and were immediately centrifuged at 2450 *g* for 15 min. A minimum volume of 10 ml of plasma was used in all cases. Primary prostaglandins were extracted, after acidification with citric acid at pH 3, with ethyl acetate/cyclohexane (1 : 1). Then the extract was chromatographed on a silicic acid column. The eluted fractions corresponding to PG E's were

dried and dissolved in 0.6 ml of the buffer used for all solutions (gelatin phosphate buffer saline : 0.1 M phosphate pH 7.4 0.9% NaCl, 0.1% sodium azide and 0.1% gelatin). A sample of 0.2 ml for estimation of recovery and two others of 0.1 ml for PG E_1 radioimmunoassay with each of two rabbit and sheep antisera were withdrawn. The mixture containing 0.1 ml [^{3}H] PG E_1 ($\simeq$ 7500 dpm), 0.1 ml unknown sample or standard PG E_1 (5 to 300 pg) and 0.1 ml of an appropriate antiserum dilution, was incubated 2 hr at 4°C although equilibrium was attained after 30 min. One ml of cold dextran charcoal was then added. Ten minutes later the tubes were centrifuged at 4°C for 5 min at 2000 *g*, the supernatant fluid was decanted into plastic vials and 10 ml Instagel (Packard) were added. Counting efficiency in an 'Intertechnique' Spectrometer was 27%.

3. Results

3.1. *Antisera binding parameters*

3.1.1. Scatchard plots

The average association constant $K_{a(av)}$ and the antibody concentration (n = number of sites) of each

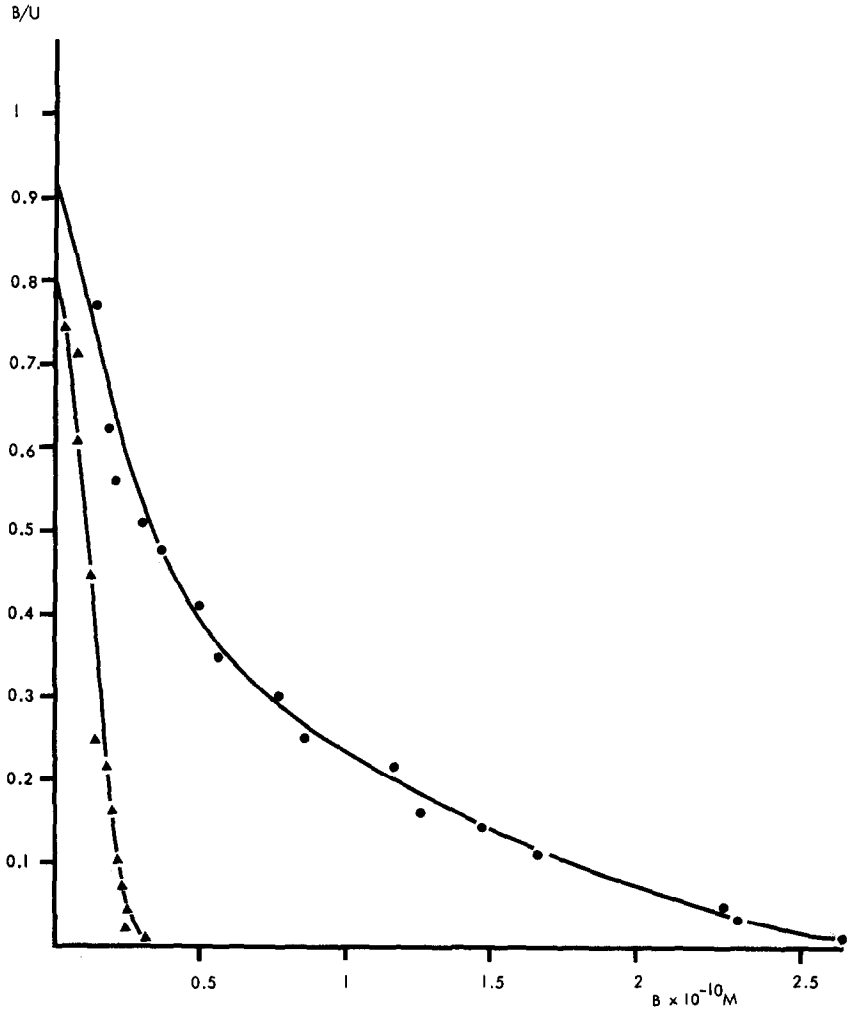

Fig.1. Scatchard plots for the PG E_1 –antibody complex at 0°C performed with (a) rabbit antiserum, 1 : 30 000 dilution (●——●) and (b) sheep antiserum, 1 : 50 000 dilution (▲——▲). Dextran coated charcoal was used for the separation of bound (B) from free (U) fractions.

antiserum were calculated from a Scatchard plot of the data (fig.1) : rabbit PG E_1 antiserum # A/79 585 $K_{a(av)} = 4.8 \times 10^8$ M^{-1}, n = 5×10^{-10} M: sheep PG E_1 antiserum # M 144 $K_{a(av)} = 1.9 \times 10^{10}$ M^{-1}, n = 1.5×10^{-11} M.

3.1.2. Dose-response curves

Figs. 2 and 3 show the inhibition curves established for various prostaglandins with [^{3}H] PG E_1 as tracer; the dilution of each antiserum was calculated to bind about 40% of same total radioactivity added (Bo). In accordance with the $K_{a(av)}$ values, 5 pg of PG E_1 gave a 15% binding decrease with sheep antiserum (fig.3) whereas 15 pg were necessary to obtain the same decrease with the rabbit antiserum (fig.2).

3.1.3. Specificity

Some aspects of the specificity of the two antisera are shown in figures 2 and 3 as well. Most striking is the complexity of the inhibition curves with PG B_1 and PG B_2 with the rabbit antiserum (fig.2), whereas PG B_1 and PG A_2 do not inhibit the sheep antiserum. The cross reaction with PG E_2 was approximately the same with both antisera.

3.2. *PG* E_1 *Values of human peripheral plasma as assayed with the rabbit and sheep antisera*

3.2.1. Comparative study with the same plasma samples

Assays were performed with extracts of 10 ml or more of plasma. As can be seen in table 1 striking differences were found for the 19 samples tested. The mean value ± SD was 35.8 ± 11.3 pg/ml with rabbit antiserum and 2.6 ± 1.9 pg/ml with sheep antiserum. With the sheep antiserum, PG E_1 was undetectable (< 1.5 pg/ml) in 6 cases. The value for such a sample was considered as zero for calculation of the mean value and the standard deviation.

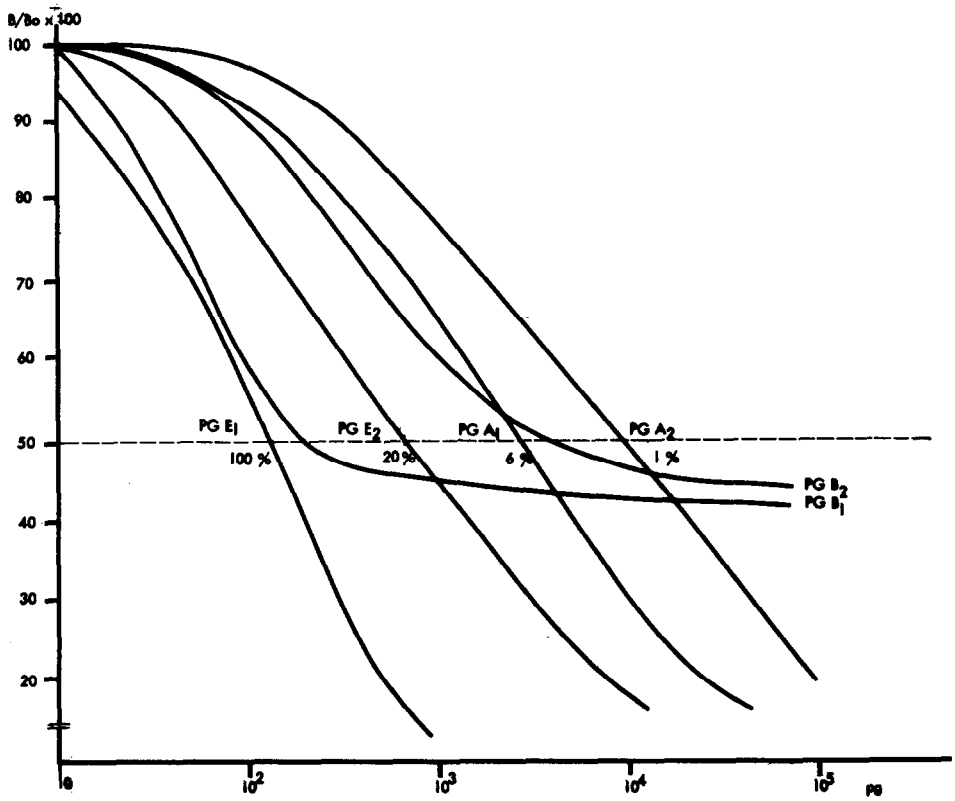

Fig.2. Binding inhibition of [^{3}H] PG E_1 to rabbit PG E_1 antiserum by various prostaglandins. Final dilution employed as 1 : 24 000 for 40% binding (Bo).

3.2.2. Demonstration of 'PG E_1-like' material in the assay using rabbit antiserum

In order to explain the relatively high levels obtained with the rabbit antiserum, 30 ml of plasma were submitted to the same purification procedure, except that the extract was resuspended in 1.5 ml of gelatin phosphate buffer saline. An aliquot of 0.3 ml was used for estimation of recovery and 0.1 ml (in duplicate) was used for radioimmunoassay with each antiserum (table 2 A-1); 3 pg/ml plasma were measured with the sheep antiserum and 105 pg/ml with the rabbit antiserum. To 0.7 ml of the residual volume sheep antiserum was added in excess (final dilution, 1 : 4000 instead of 1 : 36 000) and the mixture was incubated 2 hr at 4°C. Then 20 μl of sheep anti rabbit Fab (gift of Dr Cazenave, Institut Pasteur) were added and the mixture left overnight at 4°C, thus allowing for complete precipitation of bound PG E_1. After centrifugation (30 min at 2500 *g*), the supernatant fluid was examined for its PG E_1 content by radioimmunoassay with each antiserum (table 2 B-1) : no detectable PG E_1 was found with sheep antiserum whereas 97 pg/ml were obtained with rabbit antiserum.

Under the same conditions, a standard solution of PG E_1 (150 pg) was used to verify the binding capacity of each antiserum for this prostaglandin (table 2, A-2 and B-2). A water blank was submitted to the same procedure (table 2, A-3 and B-3).

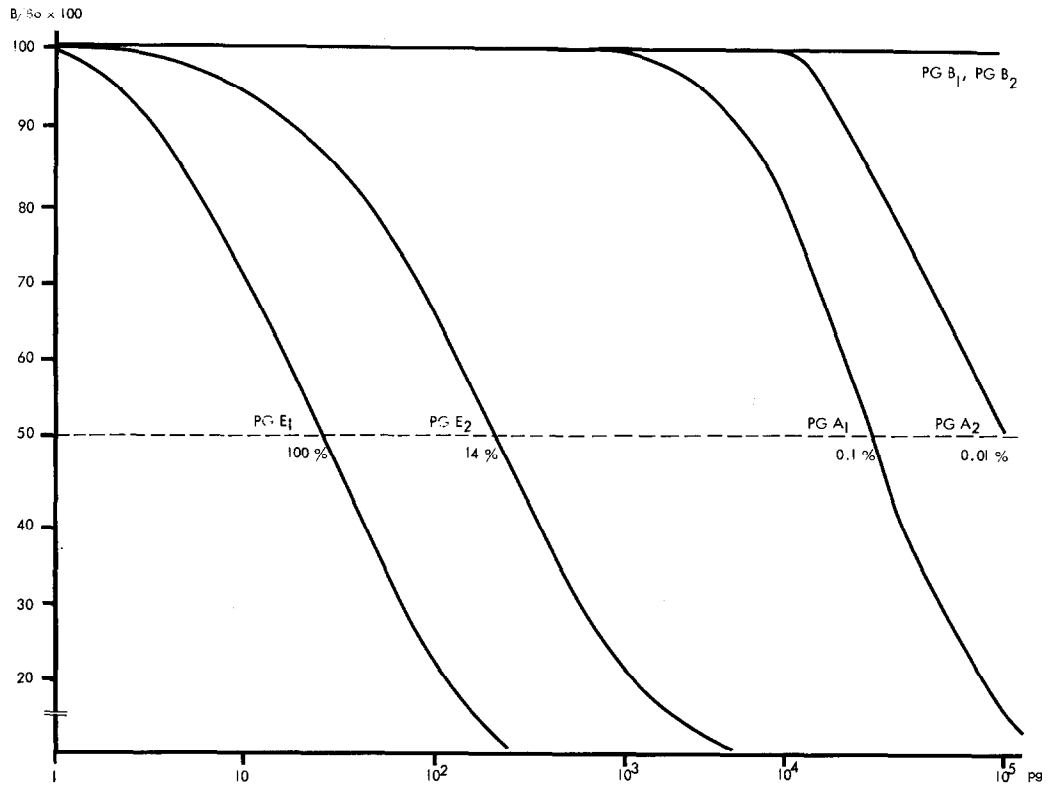

Fig.3. Binding inhibition of [^{3}H] PG E_1 to sheep PG E_1 antiserum by various prostaglandins. Final dilution employed as 1 : 45 000 for 40% binding (Bo).

Table 1
Comparative study of the values of 'PG E_1' measured in 19 plasma samples with rabbit and sheep PG E_1 antisera

	Amount of 'PG E_1' (pg/ml)	
	Rabbit antiserum	Sheep antiserum
1	46	8
2	42	4
3	53	5
4	40	2
5	38	2
6	43	4
7	43	5
8	40	3
9	43	nd[a]
10	42	2
11	26	3
12	27	4
13	30	3
14	18	nd
15	18	nd
16	51	nd
17	38	4
18	23	nd
19	20	nd
Mean ± SD	35.8 ± 11.3	2.6 ± 1.9

[a] nd : not detectable.

4. Discussion

It has been suggested that the difficulty encountered in raising specific antibodies against the primary prostaglandins (except for PG Fα) [5,7] is due to the instability of PG E to chemical coupling procedures or to enzymes (dehydrases and isomerases) found in the blood of various species [13,14]. Some investigators raised specific antisera in rabbits by immunizing with PG E_1 or PG A_1 conjugated to poly-L-Lysine and then adsorbed on R 36 strains cells *Pneumococcus*, or with PG E_1 and PG E_2 conjugated to thyroglobulin; they suggested that the hapten was protected against catalytic activity using different carriers. Our results show clearly that the sheep immunized against PG E_1-BSA raised antibodies with high affinity for and directed mainly against PG E_1 while all the five rabbits immunized against the same immunogen and with the same schedule of immunization raised antibodies that reacted with PG B_1 as effectively. It appears that chemical dehydration of PG E_1 during the coupling procedure did not occur. It is tempting to ascribe the differential specificity of PG E_1 antibodies to a differential enzymatic activity : e.g. PG A isomerase was detected in rabbit but not in sheep serum. However with PG E_2-BSA as immunogen, the same dehydrase and isomerase activities were not observed in rabbit; the specificity was directed toward PG E_2 and PG B_2 did not react appreciable. The possibility that a partic-

Table 2
Demonstration of 'PG E-like' material in the assay using rabbit PG E_1 antiserum

	Immunoreactive material as PG E_1 (pg)			
	A		B	
	Rabbit antiserum	Sheep antiserum	Rabbit antiserum	Sheep antiserum
1 - Plasma extract (per ml)	105	3	97	0
2 - PG E_1 standard (150 pg)	144	147	0	0
3 - Phosphate buffer	0	0	0	0

A : After silicic acid chromatography of the plasma.
B : After specific withdrawal of bound PG E_1 from the extract (see text), 'PG E' material was still measured with the rabbit antiserum.

ular conformation of the hapten molecule promotes the recognition by the immunocyte of a structure similar to that of PG A and/or PG B must be considered. In order to investigate this hypothesis fractionation of rabbit PG E_1 antibody populations is in progress.

Two years ago, we published [15] the first reported low values for plasma PG Fα (1+2) ($\simeq$ 10 pg/ml) and PG E_1 ($\simeq$ 40 pg/ml) and, more recently [11], for PG E_2 ($\leqslant$ 5 pg/ml), obtained by radioimmunoassay after extraction and silicic acid chromatography. We assumed that our results rested upon the volume of plasma used ($\simeq$ 10 ml) and the care taken in collecting the blood and handling of the plasma in order to prevent release and/or synthesis of PGs by blood cells. The lower values of PG E_1 found with sheep PG E_1 antiserum emphasize a third major factor; the quality of PG antisera. The discrepancy between the values of PG E_1 obtained with the two different PG E_1 antisera might be related to the low affinity and poor specificity of the rabbit antiserum which measures (table 2) PG E_1 and other related substances: possibly PG metabolites or any 'PG E_1-like' structure. The values we find for the primary PGs are in agreement with those calculated by Samuelsson [17]. Sometimes PG E_1 was not even detectable in our plasma samples (in 6 of 19 PG E_1 determinations) (table 1). This fact is consistent with the postulate that PGs from tissues into circulating blood and/or from blood cells (namely platelets) are being released intermittently into plasma. Thus, primary PG levels in peripheral plasma may have little significance in basal conditions, but could be informative in appropriately stimulated systems, for example in some physiological and pathological conditions.

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