ON THE SPECIFICITY OF ANTISERA AGAINST PROSTAGLANDINS A_2 AND E_2

A. JOBKE, B.A. PESKAR
Pharmakologisches Institut der Universität, 78 Freiburg i.Br., W. Germany

and

B.M. PESKAR
Extraordinariat für Gastroenterologie der Universität, A-1090 Wien, Austria

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1. Introduction

In recent years an increasing number of reports have dealt with the production and the specificity of antisera against prostaglandins (Pg) [1–9]. The antisera exhibited specificity for both the cyclopentane ring structure and the degree of unsaturation of the side chains. However, while the specificity of antisera against prostaglandins of the F series was considerable [2, 3, 6–9] and $PgF_{1\alpha}$ and $PgF_{2\alpha}$ could be differentiated by radioimmunoassay [10], antisera against prostaglandins of the A and E series often showed strong cross-reactions with prostaglandins A, B and E [1–4, 11]. In fact, several investigators [2, 3] have suggested that it would be impossible to obtain sufficiently specific antibodies against PgE and PgA. Only recently the production of more specific antisera against PgE_1 [11, 12] and PgA_1 [5] has been described. However, there is also much interest in the specific measurement of the dienoic prostaglandins PgE_2 PgA_2, since they seem to be widely distributed in biological material. Recently Zusman et al. [4] attempted to produce specific antibodies to PgE_2, but obtained antibodies directed mainly against PgA_2 with considerable cross-reactions with PgA_1 (53%), PgE_2 (26%) and PgE_1 (19%). This paper describes the specificity of antisera against PgA_2 and PgE_2.

2. Materials and methods

Prostaglandins A_1, A_2, E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ (Upjohn Co.) were a generous gift of Dr. S. Spector, Nutley, N.J. PgE_1-5,[^{3}H]6 (specific activity 68.5 Ci/mmole) and PgA_1-5,[^{3}H]6 (specific activity 132 Ci/mmole) were bought from New England Nuclear Co. [^{3}H]PgB_1, PgB_1 and PgB_2 were prepared according to the method of Zusman [13] from [^{3}H]PgE_1, PgE_1 and PgE_2 respectively. The antigens for immunization of the rabbits were synthetized by the method of Goodfriend et al. [14], using 7.5 mg bovine serum albumin (BSA), 4 mg 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (CDI) and 4 mg PgE_2 or PgA_2 respectively. After incubation at room temperature overnight the preparation was dialyzed exhaustively against many changes of distilled water. 250 μg of antigen in 0.5 ml saline were emulsified with an equal volume of complete Freund's adjuvant and injected into the toe pads of rabbits (3 per group), followed by 100 μg one and three weeks after the starting dose. The rabbits were bled 10–14 days after booster injections and the sera checked for the presence of antibodies using agar diffusion plates and microcomplement fixation [15]. Radioimmunoassay was performed as described previously [16], using charcoal for separation of the free and antibody-bound fractions.

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3. Results

All rabbits immunized with the prostaglandin immunogens not only produced antibodies against the backbone molecule BSA as shown by agar diffusion plates and microcomplement fixation, but also against the prostaglandins. The presence of the latter antibodies was shown by microcomplement fixation and the specific binding of tritiated prostaglandins by sera from the immunized animals as compared to the binding by a pool of normal rabbit sera or by preimmunization sera from the same animals.

Fig. 1 shows titration curves of 2 rabbit sera, obtained about 2 months after the first injection of PgE_2–CDI–BSA (left side) or PgA_2–CDI–BSA (right side), using three different labelled prostaglandins as antigens. For these experiments equal specific activities of the labelled compounds were used and the results were corrected for the minimal binding shown by normal rabbit sera. While the titration curves for $[^3H]PgA_1$ and $[^3H]PgB_1$ are similar for both sera, only the anti-PgE_2 antiserum binds $[^3H]PgE_1$. Fig. 2 shows, that this binding of $[^3H]PgE_1$ is rather specific. Of all the prostaglandins checked PgE_2 was most effective in displacing $[^3H]PgE_1$ from the antibody (50% displacement with 1.6 moles), followed by PgE_1 (50% displacement with 2.9 pmoles) and PgA_2 (50% displacement with 16.5 pmoles). The effect of PgA_1, PgB_1, PgB_2, $PgF_{1\alpha}$ and $PgF_{2\alpha}$ in the dose range used was comparably small.

However, using the same antiserum and $[^3H]PgA_1$ or $[^3H]PgB_1$ as the labelled antigen, the specificity of the antiserum seems changed (table 1). $[^3H]PgA_1$ is displaced most effectively by PgA_2 and $[^3H]PgB_1$ by PgB_2. PgE_2 was almost ineffective as an inhibitor in both reactions.

Fig. 3 shows the specificity of the reaction between $[^3H]PgA_1$ and the serum obtained from a rabbit which had been immunized with PgA_2–CDI–BSA. While the PgE and PgF compounds in the dose range used do

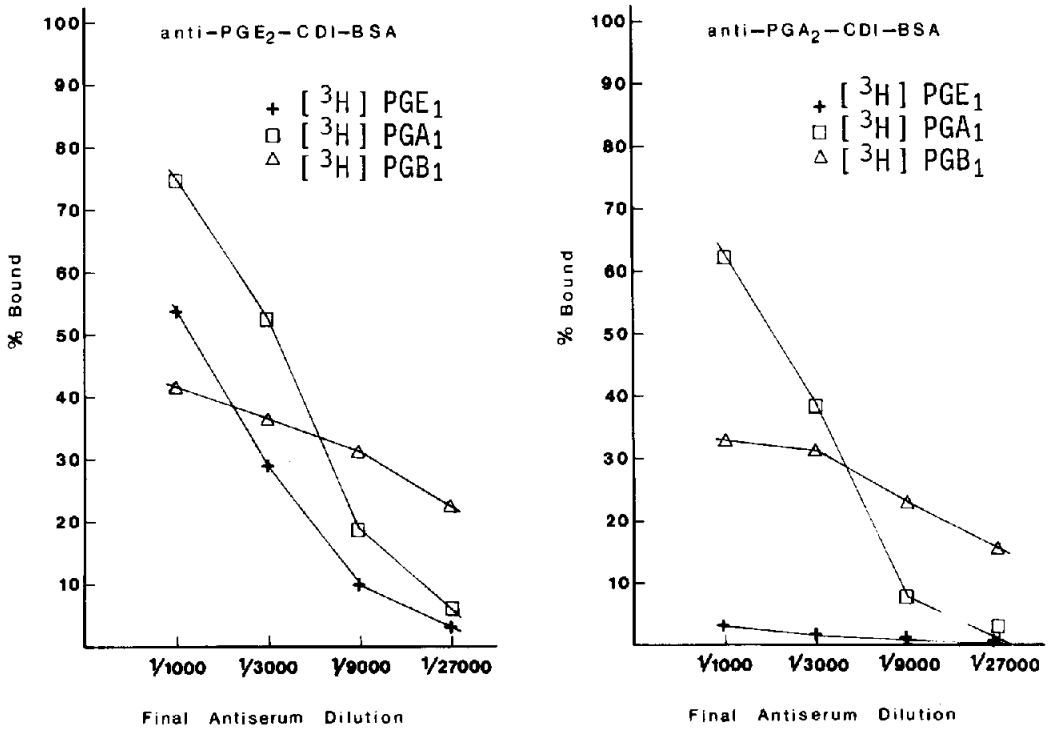

Fig. 1. Binding of $[^3H]PgA_1$, $[^3H]PgB_1$ and $[^3H]PgE_1$ by serum obtained from rabbits immunized with PgE_2–CDI–BSA (left side) or PgA_2–CDI–BSA (right side). 2000 cpm of the labelled compounds were used at equal specific activity (68.5 Ci/mmole) and the curves corrected for the binding by normal rabbit serum.

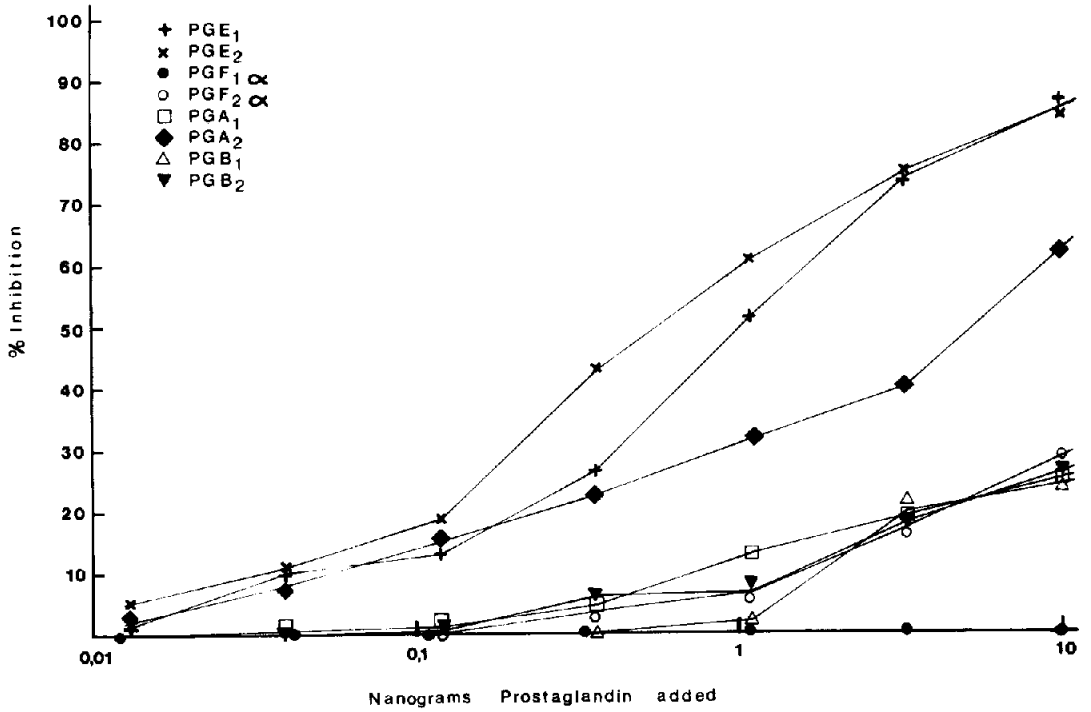

Fig. 2. Inhibition of [^{3}H]PgE_1–anti-PgE_2 binding by various prostaglandins. Final antiserum dilution employed was 1:1600.

not inhibit the reaction, both PgA and PgB compounds effectively displace [^{3}H]PgA_1 from the antibody, the dienoic compounds PgA_2 and PgB_2 (50% displacement with 1.55 pmoles and 1.7 pmoles respectively) being more effective by a factor of almost 3 than PgA_1 and PgB_1 (50% displacement with 4.0 pmoles and 4.7 pmoles respectively).

4. Discussion

Our results show, that the antisera obtained after immunization of rabbits with PgA_2–CDI–BSA or PgF_2–CDI–BSA are specific not only for the cyclopentane ring structure, but also for the degree of unsaturation of the side chains. The homologous PgE_2

Table 1

Specificities of anti-PgE_2 antiserum depending on the labelled antigen used. Final antiserum dilutions were 1:4000 for [^{3}H]PgA_1 binding, 1:10 000 for [^{3}H]PgB_1 binding and 1:1600 for [^{3}H]PgE_1 binding.

Inhibitor	50% displacement of [^{3}H]PgA_1	[^{3}H]PgB_1	[^{3}H]PgE_1
PgA_2	0.8 pmoles	> 30 pmoles**	16.5 pmoles
PgB_2	3.6 pmoles	2.4 pmoles	> 30 pmoles†
PgE_2	> 30 pmoles*	> 30 pmoles***	1.6 pmoles

* 36% Inhibition with 30 pmoles.
** 43% Inhibition with 30 pmoles.
*** 8% Inhibition with 30 pmoles.
† 26% Inhibition with 30 pmoles.

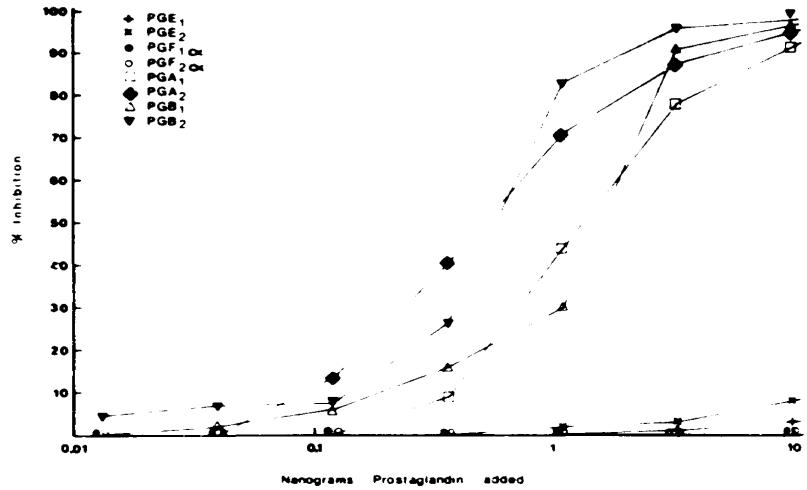

Fig. 3. Inhibition of [^{3}H]PgA_1 - Anti-PgA_2 binding by various prostaglandins. Final antiserum dilution employed was 1:3000.

bind better to the anti-PgE_2 antiserum than PgE_1, and PgA_2 binds better to the anti-PgA_2 antiserum than PgA_1. The view, that the 5,6 double bond is recognized by the antibodies is supported by the fact, that cross-reactions with other dienoic prostaglandins are stronger than with the corresponding mono-unsaturated compounds. So PgA_2 is a better inhibitor than PgA_1 in the PgE system (fig. 2) and PgB_2 is a better inhibitor than PgB_1 in the PgA system (fig. 3). Probably the specificity of our PgA_2- and PgE_2-radioimmunoassays could be even increased by using radioactively labelled PgA_2 and PgE_2 antigens instead of the heterologous [^{3}H]-PgE_1 and [^{3}H]PgA_1.

Levine et al. [17] have identified PgE_2 in tissue culture medium, by reducing PgE with borohydride and analyzing the resulting PgF in their $PgF_{2\alpha}$–anti-$PgE_{2\alpha}$ and $PgE_{1\alpha}$–anti-$PgF_{1\alpha}$ systems. The simultaneous use of our antisera against PgA_2 and PgE_1 [10, 12] and anti-PgA_1 [5] antisera should provide a simple means to differentiate between PgE_1 and PgE_2 or PgA_1 and PgA_2 respectively.

Obviously immunization with PgA_2 antigen has led to the formation of anti-PgA_2 and anti-PgB_2 antibodies and immunization with PgE_2 antigen to the formation of anti-PgE_2, anti-PgB_2 antibodies, the different antibody populations having different titers and specificities. The occurrence of anti-PgB antibodies after immunization with PgA antigens and of anti-PgA and anti-PgB antibodies after immunization with PgE antigens is in accordance with the results of other laboratories [1–4] and has been explained by the instability of PgE to chemical coupling reactions and the action of prostaglandin dehydrase [18] and isomers [19, 20]. However, Raz and Stylos [10] and Jaffe et al. [12] have obtained specific anti-PgE_1 antibodies and our results show, that it is also possible to obtain specific anti-PgE_2 antibodies. The presence of anti-PgA and anti-PgB antibodies in the same serum, binding PgA and PgB better than the anti-PgE antibodies do, might contribute to the specificity of the PgE assay.

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