SPECIFICITY OF PROSTAGLANDIN A_1 ANTISERUM AGAINST PROSTAGLANDINS A_1 AND B_1 *

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1. Introduction

Recent attempts by Jaffe et al. [1] to produce antibodies towards PGA_1 have resulted in an antiserum which cross-reacted significantly with PGE_1 and PGE_2. Levine and co-workers [2] have attempted to obtain antibodies to PGE_1 in rabbits by immunizing with PGE_1–bovine serum albumin conjugates, but obtained antibodies directed mainly against PGB_1. Similar attempts by Yu and Burke [3] have resulted in the production of PGE_1 antisera which cross reacted to an equal extent or more with the A and B prostaglandins, and of PGA_1 antisera which cross reacted to a higher extent with PGB_1. Zusman et al. [4] have attempted to produce antibodies to PGE_2 but obtained antisera directed mainly against PGA_2 and with cross reactivities towards PGA_1 (53%) and PGE_2 (26%). As a result of these studies [1–4], several investigators [2, 3] have suggested that it would be very difficult, if not impossible, to obtain antibodies directed specifically against the PGE and PGA prostaglandins.

The first report on the successful production of anti-PGA_1 serum which is highly specific for PGA_1 was reported by Stylos and Rivetz [5]. These authors immunized rabbits with poly L-lysine–PGA_1 conjugate absorbed on Pneumococcus R 36A strains cells, and obtained PGA_1 antiserum which cross reacted somewhat with PGA_2 (9.7%), but only very minimally with PGE_1 (2.9%). This report describes the relative specificity of this PGA_1 antiserum towards PGA_1 and PGB_1. Also included is a discussion of the protein carrier and method of immunization as they affect the specificity of antisera raised against PGA_1 and against PGE_1.

2. Materials and methods

Prostaglandin E_1 was generously supplied by Dr. John E. Pike of the Upjohn Co. and Prostaglandin A_1 was a gift of the Ono Company, Tokyo, Japan. PGB_1 was prepared from PGE_1 by heating in 0.5 N KOH in methanol–water (1:1) at 55–58° for 30 min. The reaction mixture was then evaporated to dryness, dissolved in water, acidified to pH = 3.0 with 1 N HCl and extracted 3 times with 5 ml diethyl ether. The pooled ether extracts were washed with water to pH = 6.0–6.5, dried over Na_2SO_4 and evaporated to dryness. A recent report by Zusman [6] has indicated that the method of converting PGE_1 to PGB_1 by heating in 1 N methanolic KOH for 2–5 min at 100° may yield products other than PGB_1. The purity of the prepared PGB_1 was therefore checked spectrophotometrically. The product was found to possess the characteristic absorption spectrum of PGB_1 with an absorption maximum at 278 nm.

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Abbreviations:
PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2; PGA_1 prostaglandin A_1; PGB_1, prostaglandin B_1; $PGF_{1\alpha}$ prostaglandin $F_{1\alpha}$; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

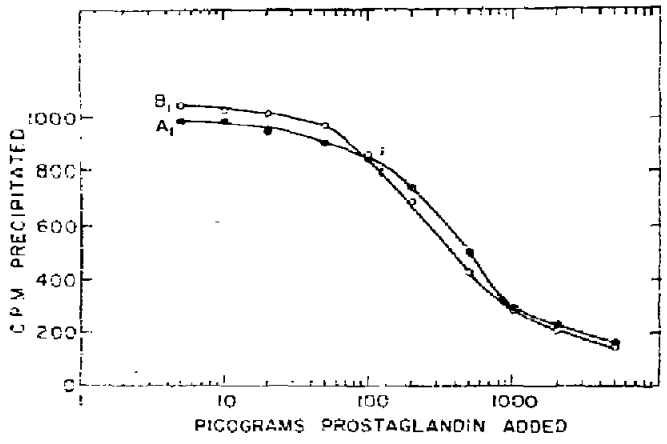

Fig. 1. Inhibition of $[^3H]PGA_1$-anti-PGA_1 binding by PGA_1 and PGB_1. Assay conditions are described in Methods. The antiserum dilution employed was 1:800 (final dilution in the assay medium was 1:4000).

PGA_1 antiserum was obtained from rabbits immunized with poly-L-lysine–PGA_1 conjugate [5]. The procedure employed in the radioimmunoassay has been described elsewhere [5].

3. Results and discussion

The PGA_1 antiserum produced in rabbits immunized with a poly-L-lysine–PGA_1 conjugate bound 50% of the added $[^3H]PGA_1$ (2000 cpm, 10 pg) at a dilution of 1:800 (final dilution of the antiserum in the assay medium was 1:4000). The standard curve of this antiserum with unlabeled PGA_1 and the cross reaction with unlabeled PGB_1 are shown in fig. 1. PGA_1 and PGB_1 appear to be equally effective in displacing antibody bound $[^3H]PGA$. These results indicate that the antibodies we obtained possess very similar binding characteristics towards PGA_1 and PGB_1. Support for this tentative conclusion was obtained from studies in which $[^3H]PGB_1$ was first added to the PGA_1 antiserum, and then displaced by varying amounts of unlabeled PGA_1 or PGB_1. The results of these experiments are given in fig. 2 and again indicate both PGA_1 and PGB_1 to be approximately equal in displacing the antibody-bound $[^3H]PGB_1$. It should be emphasized that despite the apparent inability of the antibodies produced to distinguish between PGA_1 and PGB_1, they are nevertheless specific for the

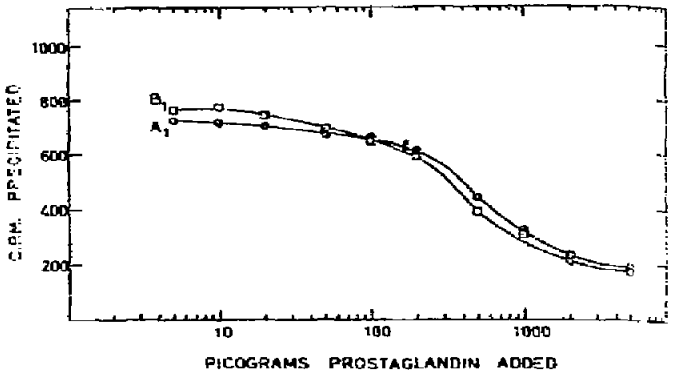

Fig. 2. Inhibition of $[^3H]PGB_1$-anti-PGA_1 binding by PGA_1 and PGB_1. Assay conditions are described in Methods. The antiserum dilution employed was 1:400 (final dilution in the assay medium was 1:2000).

cytopentene ring structure, showing only small cross reactivity with PGE_1, PGE_2, $PGF_{2\alpha}$, and $PGF_{1\alpha}$ [5]. Two conclusions can be drawn from these results. The first is that the affinity of the antibodies for initial binding of PGA_1 is stronger than towards PGB_1 since in order to achieve the same extent of binding (50%) of $[^3H]$prostaglandin, it was necessary to use a more concentrated antiserum for PGB_1 (dilution of antiserum 1:400) than for PGA_1 (dilution of antiserum 1:800). The second conclusion is that the abilities of unlabeled PGA_1 and PGB_1 to displace $[^3H]$prostaglandin (PGA_1 or PGB_1) are very similar. Taken together, these conclusions indicate to us that the antibodies we obtained were produced in response to the presence of PGA_1-containing immunogen only and are directed against PGA_1 moieties, but are not capable of distinguishing between the PGA_1 and PGB_1 structures. Previous studies [2, 3, 7] have indicated that the presence or absence of the hydroxyl groups at C_9 and C_{11} and the keto group at C_9 are the major factors in producing the specific immunogenic response against the particular prostaglandin molecule. The cyclopentene ring in both PGA_1 and PGB_1 is planar although structural differences between these two prostaglandins do exist (e.g. the orientations of the C_8 and C_{12} side chains). These differences appear however to be too small to affect significantly the overall binding affinities of anti-PGA_1 antibodies towards PGA_1 as compared to PGB_1.

The formation of antibodies directed against PGA_1 following immunization with PGA_1-containing

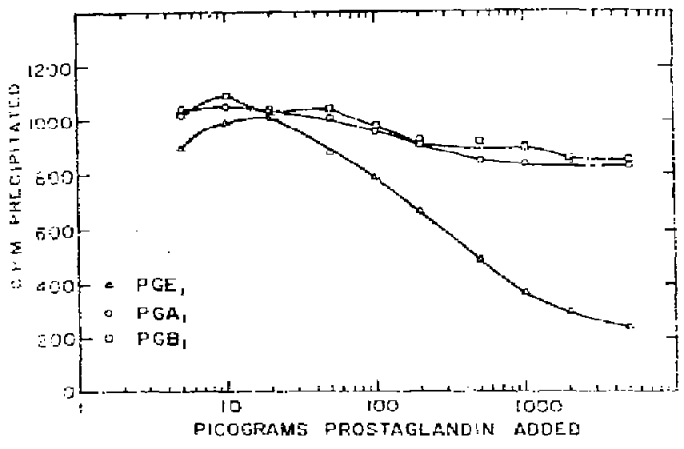

Fig. 3. Inhibition of [^{3}H]PGE_1-anti-PGE_1 binding by PGE_1, PGA_1 and PGB_1. Assay as described in Methods. The antiserum dilution employed was 1:1200.

immunogen is in contradistinction to the results of Jaffe et al. [1], Levine et al. [2] and Yu and Burke [3]. Levine and co-workers [2] have attempted to produce antibodies to PGE_1 by immunizing rabbits with PGE_1-poly-L-lysine-succinylated hemocyanin. The antibodies they obtained were however directed mainly against PGB_1 with weaker binding of PGA_1 and negligible binding of PGE_1. To account for these results, they proposed that PGE_1 was converted to PGA_1 during the carbodiimide conjugation reaction, so that the conjugate used for immunization contained PGA_1 rather than PGE_1. They further propose that prostaglandin isomerase activity, originally demonstrated by Jones in cat plasma [8] and more recently also in plasma of rabbit, dog and rat [9, 10] catalyzes the *in vivo* conversion of protein-bound PGA_1 to PGB_1 yielding an immunogen containing PGB_1 moieties. Support for this suggested mechanism was reported by Yu and Burke [3] who obtained PGE_1 antiserum which cross reacted to an equal or more extent with PGA_1 and PGB_1. They also obtained PGA_1 antiserum which cross reacted to a higher extent with PGB_1. The data presented here, while not excluding the possibility that such a mechanism is operative, indicates that the method of immunization as reported by Stylos and Rivetz [5] in which the poly-L-lysine-PGA_1 conjugate was absorbed to the Pneumococcus cells, prevented a possible conversion of PGA_1 to PGB_1. In addition it was recently possible to obtain a PGE_1 antiserum with high specificity towards PGE_1*. Since both Levine et al. [2] and Yu and Burke [3] have suggested that production of antibodies against E type prostaglandins without considerable cross-reactivity with the A and B prostaglandins would be very difficult if not impossible, we were interested in determining the cross reactivities of the PGE_1 antiserum against PGA_1 and PGB_1. These results are given in fig. 3, and indicate that neither PGA_1 nor PGB_1 could displace 50% of the [^{3}H]PGE_1 initially bound. Accurate evaluation of the cross reactivities of these prostaglandins were therefore not possible; the cross reactivities are however less than 1%. Of special significance is the fact that the binding affinities of PGA_1 and PGB_1 towards the PGE_1 antibodies, however small, are nevertheless very similar. These results are in agreement with those obtained earlier (fig. 1 and 2) with regard to the relative binding affinities of PGA_1 and PGB_1 towards PGA_1 antiserum.

A radioimmunoassay for prostaglandins provides a simple technique for the quantitative determination of these compounds in biological fluids and tissues. An absolute necessity for the development of a specific radioimmunoassay is the production of antibodies capable of distinguishing between the various structurally related prostaglandins. Stylos and Rivetz [5], by choosing a suitable protein conjugate in combination with a second carrier (Pneumococcus cells) were able to prevent the possible conversion of PGA_1 to PGB_1 and thus obtain a PGA_1 antiserum directed mainly against PGA_1. Furthermore, the data presented here on the cross reactivities of the PGE_1 antiserum with PGA_1 and PGB_1 (fig. 3) indicates that the chemical conversion of PGE_1 to PGA_1 and the enzymatic conversion of PGA_1 to PGB_1 as suggested by Levine et al. [2] and Yu and Burke [3], was blocked. It therefore appears that the type of protein carrier used for conjugation and the method of immunization are the major determinants which affect the resulting specificities of the antibodies produced, and that by proper selection of these determinants, it is possible to obtain specific antibodies against prostaglandins E or prostaglandin A compounds.

* The PGE_1 antiserum was prepared at the Worcester Foundation. Manuscript dealing with the method of preparation and detailed immunological specificity is in preparation.

Acknowledgements

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