CHARACTERIZATION OF ANTIBODY TO PROSTAGLANDIN $F_{2\alpha}$*

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Summary: An antibody that reacted with $PGF_{2\alpha}$ was produced in two species (goat and rabbit) by injection of a Prostaglandin $F_{2\alpha}$-Bovine Serum Albumin conjugate. Antibody binding was altered most by structural changes in the cyclopentane ring, and changes at the C-13,15 positions of the prostaglandin molecule. Incubation of standard solutions, or test serum with a prostaglandin dehydrogenase enzyme decreased the immuno-reactive activity. Parallel dose responses were obtained with biological fluids and prostaglandin standards. Of the naturally occurring prostaglandins tested, only $PGF_{1\alpha}$ cross-reacted significantly. The antibody described should be a useful tool for developing an immunoassay to use in elucidating the physiological and pharmacologic functions of prostaglandin $F_{2\alpha}$.

Radioimmunoassays have now been developed to measure diverse kinds of molecules. Larger antigenic carriers have been used to render small non-antigenic molecules antigenic. With this background, an attempt was made to develop an immunoassay for prostaglandin $F_{2\alpha}$. Prostaglandins are 20 carbon, unsaturated fatty acids that have been shown to possess a wide variety of pharmacological activities. In general, they are very

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potent smooth muscle stimulants, and intracellular modulators of hormonal action. Their extreme potency, and short biological half-life in peripheral circulation dictate the use of very sensitive, precise analytical methods to quantitate physiologically active concentrations in biological fluids and tissue samples.

This correspondence describes the preparation and partial characterization of an antibody produced to a bovine serum albumin-prostaglandin $F_{2\alpha}$ conjugate, which was developed primarily as an immunoassay for $PGF_{2\alpha}$.

Prostaglandin $F_{2\alpha}$ was coupled to bovine serum albumin by a modification of the method described by Lieberman et al. (1). One hundred mg of $PGF_{2\alpha}$ and 0.065 ml of tri-n-butylamine were dissolved in 2.77 ml of dioxane, cooled to 10°C, and 0.034 ml of isobutyl chlorocarbonate added. The mixture was allowed to react at 4°C for 20 minutes, then added to 20.3 cc of 1:1 water-dioxane containing 385 mg of BSA and 0.38 ml of 1 N NaOH. The mixture was stirred for four hours at 4°, then dialyzed against running water for 18 hours and the pH adjusted to 4.5 with 1 N HCl. The precipitate was collected, suspended in 100 ml of water and redissolved with a minimal amount of $NaHCO_3$. The clear solution was lyophilized and stored frozen until used for immunizations.

The prostaglandin-protein conjugate was diluted to a concentration of 1 mg/cc with distilled water and emulsified with an equal volume of Freund's adjuvant. One goat and ten New Zeeland rabbits were injected at weekly intervals for three weeks, and then at 3 or 4 week intervals. The goat was injected with 3 mg in multiple sites along the flank area, and the rabbits with 1 mg in the footpads (2 injections) then subcutaneously in the scapular area. Blood serum was collected one week after the fourth immunizing injection, and at monthly intervals thereafter. The goat and each of the rabbits produced antibody which bound ^3H-labeled prostaglandin. A detectable antibody titer was present after

3 injections in some animals. Affinity of antibody binding was determined by incubating ^{3}H-$F_{2\alpha}$ (Sc 14.3 c/mM) with diluted primary antisera, then separating antibody bound from free ^{3}H by incubation with a second antibody generated against γ-globulin of the species immunized. Specificity of the antibody was quantitated by determining the amount of an unlabeled structurally related molecule necessary to displace ^{3}H-$F_{2\alpha}$ from the primary antibody.

Changes in affinity of the antibody caused by alterations of the prostaglandin molecule are illustrated in Fig. 1. As expected, changes in substituents, or the configuration of substituents, on the pentane ring or at the C 13-15 portion of the molecule influenced antibody affinity the most. Of the known endogenous prostaglandins tested, only $PGF_{1\alpha}$

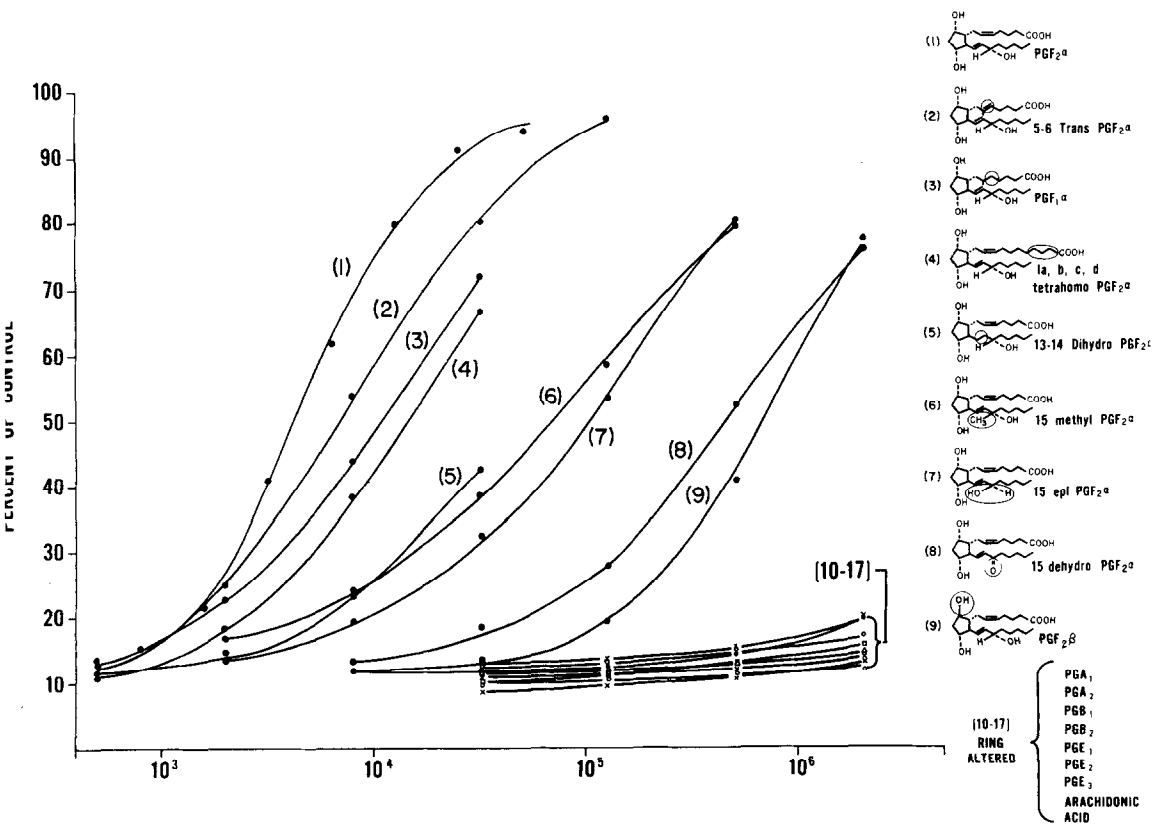

Figure 1. Specificity of $PGF_{2\alpha}$ Antiserum.

interacted appreciably with the antibody (17%). The antibody had less than 1% of the affinity for the metabolites tested, 15-keto $F_{2\alpha}$ and 13, 14 dihydro-15 keto $F_{2\alpha}$, when compared to $F_{2\alpha}$.

Maximal sensitivity was defined as that amount of antigen necessary to significantly alter the amount of antibody bound antigen. Fifty picograms was significantly different than zero in 14 of 15 replicate experiments, using an antibody dilution of 1:2000 and adding 1500 cpm of ^{3}H-$F_{2\alpha}$/assay tube. Higher affinity antibody obtained from subsequent bleedings indicate a possible maximum sensitivity of 10-25 picograms.

Other experiments were performed to characterize the antibody, and determine validity of its use as a tool for prostaglandin radioimmunoassays. Incubation of solutions with known concentrations of $PGF_{2\alpha}$ or unknown test sera with a prostaglandin dehydrogenase enzyme resulted in diminution of the activity measured by immunoassay (Table 1). Also incubation of ^{3}H-$PGF_{2\alpha}$ with dehydrogenase decreased antibody affinity for the prostaglandin, as expected. Specificity of the dehydrogenase (2) confirms that activity measured by immunoassay was prostaglandin.

Table 1. Reduction of prostaglandin activity by incubation with a prostaglandin dehydrogenase enzyme.

	Potency*(ng/ml)	
Sample **	A	B
1	14	<4†
2	60	<4
3	78	21
4	124	37

* A - prior to incubation, B - following incubation with enzyme.

**Sample #1 and 2 - standard solutions of $PGF_{2\alpha}$. Sample #3 and 4 - test serum.

†Below lower limit of sensitivity of the assay.

Validity of the assay was further demonstrated by comparing the slope of dose-response curves obtained with prostaglandin standards and serial dilutions of unknown test sera (Fig. 2). The parallelism obtained indicates a similar antibody affinity for the immuno-reactive material in test sera and for the standard prostaglandin solutions. It also indicates that potency, determined by immunoassay, should be independent of the volume of test sera added.

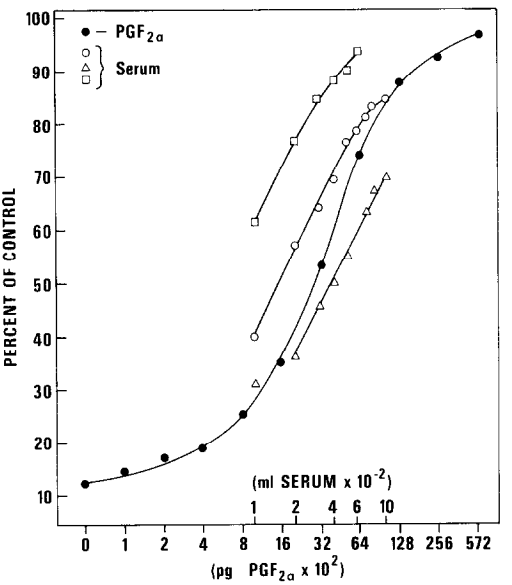

Figure 2. Parallelism of dose-response obtained with standard solutions and serial dilutions of test sera.

Antibody affinity was determined by calculation of the ratio of bound to free antigen at various antigen concentrations, assuming an antibody valence of 2, and with a calculated (3) antibody concentration of 2.3 X $10^{-6}M^{-1}$. The K thus calculated was 2.75 X 10^{-9} $\frac{1}{M}$. Indistinguishable binding curves were observed when either the labeled or unlabeled antigen was added first, or when both species of antigen were added to antibody simultaneously. These data are suggestive of an equilibrium system; further experiments indicated that the equilibrium of the primary antibody was obtained within 30 minutes. However, when using a second-anti-

body immunoassay, potency estimates of serum indicated a final incubation of 48 hrs was necessary following addition of second antibody to the reaction. The position of equilibrium was temperature dependent, in that a shift to a higher concentration of unbound antigen was obtained at 23°C, than at 5°C.

Endogenous concentrations of prostaglandin, measured in unextracted peripheral serum samples obtained from human subjects (Fig. 3), compared favorably with other published values obtained by similar methods of analysis (4). However, recent results indicate abnormally high values measured in serum probably reflect prostaglandin synthesis and/or release

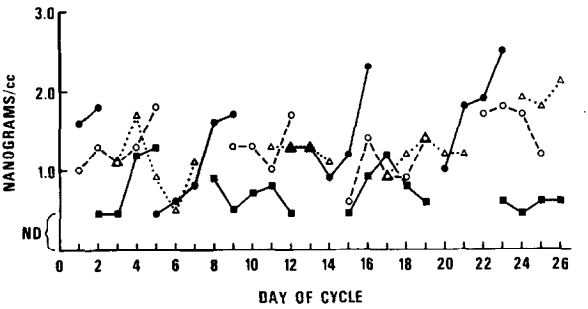

Figure 3. Serum concentrations of $PGF_{2\alpha}$ in 4 humans during a menstrual cycle.

from cellular components during the clotting process. Prostaglandin concentrations in serum and plasma of several species and values obtained in extracted and unextracted plasma are currently being determined. Disappearance of injected prostaglandin from peripheral serum indicated an initial half-life of about 1 minute (Fig. 4), also in agreement with previously published data (5).

These experiments indicate that the antibody described is capable of detecting physiological concentrations of prostaglandin $F_{2\alpha}$ in biological fluids. The antibody described should be a useful tool for developing an assay to measure endogenous concentrations of prostaglandin in biological tissues and fluids.

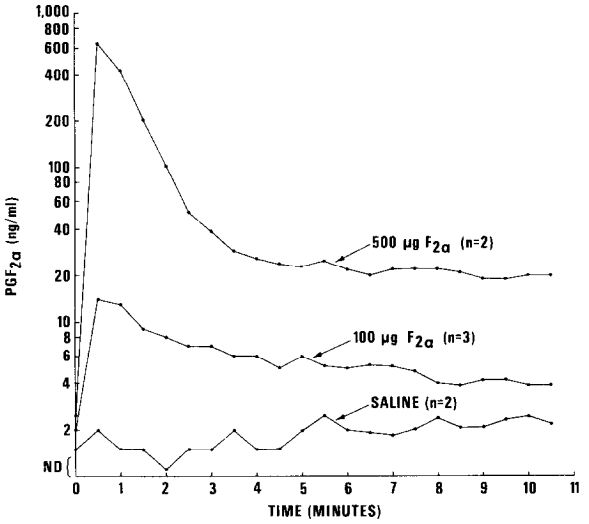

Figure 4. Serum concentrations of $PGF_{2\alpha}$ in rhesus monkeys following an I.V. injection.

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