ANTISERA AGAINST 13,14-DIHYDRO-15-KETO-PROSTAGLANDIN E_2

B. A. PESKAR and A. HOLLAND
Pharmakologisches Institut der Universität D-7800 Freiburg i.Br., W. Germany

and

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

Received 8 April 1974

1. Introduction

Increasing interest in the physiological role of the various prostaglandins (Pgs) has lead to the development of specific radioimmunoassays for many of these compounds [1–6]. However, it is well known that prostaglandins, especially of the E and F type, are rapidly inactivated by many tissues [7,8]. Therefore, the determination of Pg metabolites might often add valuable information as to total Pg release by a tissue. The first steps of enzymatic inactivation are generally oxidation of the C_{15} hydroxyl group to form 15-keto-prostaglandins and then reduction of the $C_{13}-C_{14}$ double bond. Radioimmunoassays for 15-keto-$PgF_{2\alpha}$ and 13,14-dihydro-15-keto-$PgF_{2\alpha}$ have been described recently [9–13]. 13,14-Dihydro-15-keto-PgE_2 was measured only indirectly so far, making use of its cross-reaction with an antiserum against 13,14-dihydro-15-keto-$PgF_{2\alpha}$ [14]. While 96% of the serologic activity of 13,14-dihydro-15-keto-PgE_2 was destroyed by heating at 100°C at pH 12.5 for 5 min, 13,14-dihydro-15-keto-$PgF_{2\alpha}$ was stable under such conditions. Radioimmunoassay before and after heating at alkaline pH gave an indirect estimate of the amount of the PgE_2-metabolite. This paper describes the production of specific antisera against 13,14-dihydro-15-keto-PgE_2 and their use for radioimmunoassay.

* Present address: Medizinische Universitätsklinik D-7800 Freiburg 1.Br., W. Germany

2. Materials and methods

The prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$, A_1, A_2 and the metabolites 13,14-dihydro-15-keto-PgE_2, 15-keto-PgE_2, 13,14-dihydro-15-keto-$PgF_{2\alpha}$ and 15-keto-$Pgf_{2\alpha}$ were generous gifts of Dr. J. Pike (Upjohn Co. Kalamazoo, USA). PgB_1 and PgB_2 were prepared according to the method of Zusman [15] from PgE_1 and PgE_2 respectively. PgE_2-5,6,8,11,12,14,15-H^3 (specific activity 125 Ci/mmol) was bought from New England Nuclear Co. The antigen for immunization of the rabbits was synthetized by the method of Goodfriend et al. [16], using 7.5 mg bovine serum albumin (BSA), 4 mg 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide and 3 mg 13,14-dihydro-15-keto-PgE_2. After incubation at room temperature overnight the preparation was dialyzed exhaustively against many changes of distilled water. 100 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, followed by 100 μg one week later and then at monthly intervals. The rabbits were bled 10–14 days after booster injections. Microcomplement fixation was performed as described by Levine [17].

The radioactive antigen was prepared from ^{3}H-PgE_2 as described by Anggård et al. [18], using guinea pig lung as enzyme source. Briefly, lungs of guinea pigs were rapidly excised and transferred to ice-cold Bucher medium (0.02 M KH_2PO_4, 0.072 M K_2HPO_4, 0.0276 M nicotinamide, 0.0036 M $MgCl_2$, pH 7.4)

to which 1 μg/ml indomethacin had been added. Indomethacin was added to prevent synthesis of endogenous Pgs during the following procedures. The lungs were minced and homogenized in an Ultra-Turrax homogenizer (Janke and Kunkel, KG, Staufen, Germany). After centrifugation at 900 *g* for 15 min, the supernatant was centrifuged at 105 000 *g* for 60 min. Ten μl 0.01 M NAD and 250 nmCi H^3-PgE_2 were added to portions of 1 ml of supernatant. The mixtures were incubated at 37°C for 1 hr. Incubation was stopped by the addition of 5 ml absolute ethanol. Five ml H_2O were then added and the mixtures acidified to pH 3.0 with N HCl. They were then extracted with 20 ml ether. After centrifugation the organic phase was taken off and evaporated in a stream of air. The residues containing more than 80% of the original radioactivity were dissolved in 70% ethanol. According to Anggård et al. [18] this procedure results in the formation of two PgE_2 metabolites, 13, 14-dihydro-15-keto-PgE_2 and 13,14-dihydro-PgE_2. No attempt was made to separate these two compounds.

Radioimmunoassay was performed as described previously [6], using 0.5 ml charcoal suspension (20 mg/ml) for separation of bound and free fractions.

3. Results and discussion

All three rabbits immunized with the 13,14-dihydro-15-keto-PgE_2-conjugate developed specific antibodies within a few weeks. The presence of antibodies in the sera was checked for by microcomplement fixation. The complement fixation obtained using the antiserum at a 1:600 dilution and the 13, 14-dihydro-15-keto-PgE_2-carbodiimide-BSA conjugate or BSA alone as the antigen is shown in fig. 1. While up to 80% of the complement were fixed in the presence of the specific antigen at this antiserum dilution, there was minimal fixation using the carrier protein BSA as antigen. The specificity of the fixation is further shown by inhibition experiments as illustrated in fig. 2. 13,14-dihydro-15-keto-PgE_2 is a better inhibitor in the complement fixation system than 15-keto-PgE_2. PgE_2 in amounts up to 10 μg does not inhibit the reaction at all.

The specificity of the radioimmunoassay, using H^3-PgE_2 that had been incubated with guinea pig

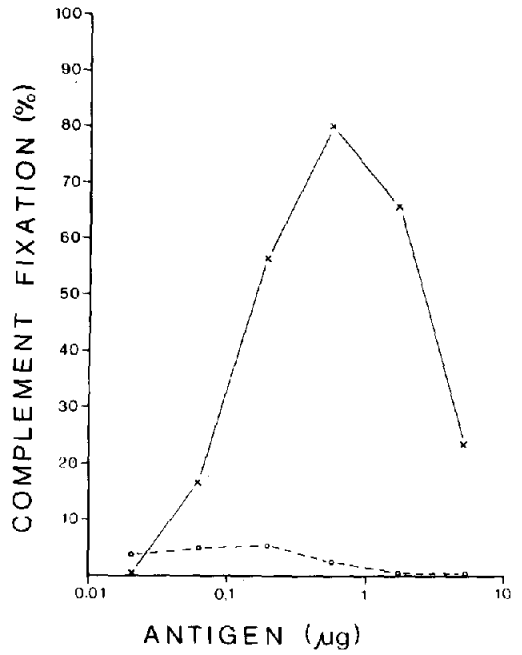

Fig. 1. Complement fixation of rabbit anti-13,14-dihydro-15-keto-PgE_2 at 1:600 dilution with 13,14-dihydro-15-keto-PgE_2-carbodiimide-BSA (X) or BSA (○).

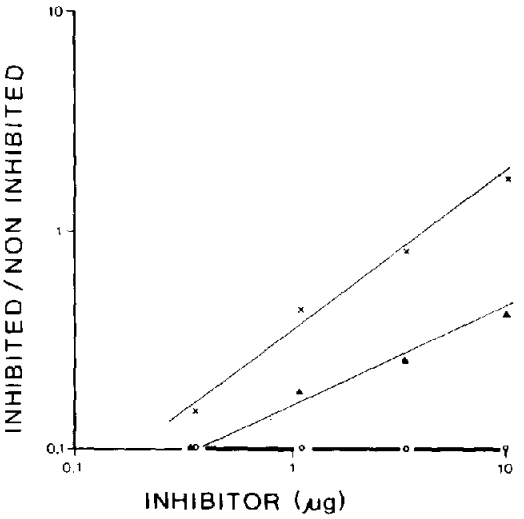

Fig. 2. Inhibition of complement fixation by 13,14-dihydro-15-keto-PgE_2 (X), 15-keto-PgE_2 (▲) and PgE_2 (○). The antiserum dilution was 1:600 and 0.55 μg of the antigen was used.

Table 1

Prostaglandin	Nanograms required to displace 50% of bound label	Relative cross-reaction (%)
13,14-dihydro-15-keto-PgE_2	3.6	100
PgA_2	80.2	4.5
15-keto-PgE_2	101.4	3.6
PgA_1	227.4	1.6
13,14-dihydro-15-keto-$PgF_{2\alpha}$	689.5	0.5
15-keto-$PgF_{2\alpha}$	> 1000.0	< 0.4
PgE_1	> 1000.0	< 0.4
PgE_2	> 1000.0	< 0.4
PgB_1	> 1000.0	< 0.4
PgB_2	> 1000.0	< 0.4
$PgF_{1\alpha}$	> 1000.0	< 0.4
$PgF_{2\alpha}$	> 1000.0	< 0.4

Specificity of antiserum against 13,14-dihydro-15-keto-PgE_2 as measured by radioimmunoassay. The final antiserum dilution was 1:1200 and 4000 cpm/tube of labelled antigen were used.

lung homogenate as the labelled antigen, is shown in table 1. The antiserum was used at a 1:1200 final dilution in these experiments. This antiserum dilution bound about 40% of the added radioactivity (4000 cpm). The best inhibitor in this system is 13,14-dihydro-15-keto-PgE_2, 3.6 ng producing 50% inhibition of binding of labelled antigen to the antiserum. The assay is linear from 400 pg up to 40 ng for this compound. 15-Keto-PgE_2, differing only by the presence of the $C_{13}-C_{14}$ double bond, shows 3.6% relative cross-reaction and PgE_2 less than 0.4%. These results indicate the immunodominance of the 15-keto group and the reduced $C_{13}-C_{14}$ double bond in the antigen molecule. This observation is further supported by the fact, that 13,14-dihydro-15-keto-$PgF_{2\alpha}$, having the same side chains as 13,14-dihydro-15-keto-PgE_2 but a different ring structure, was recognized by the antiserum to some extent, while 15-keto-$PgF_{2\alpha}$ and $PgF_{2\alpha}$ did not interfere with the assay. 13,14-Dihydro-PgE_2 was not available to us, but some cross-reaction by this compound can be expected from the above results. An antiserum against 13,14-dihydro-15-keto-$PgF_{2\alpha}$ showed a relative cross-reaction of 2.5% with the corresponding 13,14-dihydro-$PgF_{2\alpha}$ [9].

While Pgs of the E, F and B series bind only negligibly to the antiserum against 13,14-dihydro-15-keto-PgE_2, PgA_2 and PgA_1 were found to cross-react significantly. Similarly, immunization with PgE_1- and PgE_2-conjugates has lead to the production of antisera, which bind best PgA_1 and PgA_2 respectively [1,4]. Obviously the PgE ring structure in the immunogen can easily be dehydrated either chemically during the coupling procedure or enzymatically [19], thereby being transformed into the PgA ring structure. Cornette et al. [12] pointed out the advantages of a radioimmunoassay for 13,14-dihydro-15-keto-$PgF_{2\alpha}$, the biologically inactive $PgF_{2\alpha}$ metabolite. Similarly, 13,14-dihydro-15-keto-PgE_2 is a biologically inactive metabolite of PgE_2, which cannot be determined by bioassay. The radioimmunoassay described here, being sufficiently specific, is presently being used for the measurement of this compound in biological material.

References

[1] Jaffe, B. M., Smith, I. W., Newton, W. T. and Parker, C. W. (1971) Science 171, 494–496.
[2] Levine, L., Gutierrez Cernosek, R. M. and Van Vunkis, H. (1971) J. Biol. Chem. 246, 6782–6785.
[3] Yu, S. C., Chang, L. and Burke, G. (1972) J. Clin. Invest. 51, 1038–1042.
[4] Zusman, R. M., Caldwell, B. V., Speroff, L. and Behrman, H. R. (1972) Prostaglandins 2, 41–53.
[5] Kirton, K. T., Cornette, J. C. and Barr, K. L. (1972) Biochem. Biophys. Res. Comm. 47, 903–909.
[6] Jobke, A., Peskar, B. A. and Peskar, B. M. (1973) FEBS Letters 37, 192–196.
[7] Ferreira, S. H. and Vane, J. R. (1967) Nature 216, 868–873.
[8] Anggård, E. (1971) Ann. N.Y. Acad. Sci. 180, 200–215.
[9] Granström, E. and Samuelsson, B. (1972) FEBS Letters 26, 211–214.
[10] Levine, L. and Gutierrez Cernosek, R. M. (1973) Prostaglandins 3, 785–804.
[11] Stylos, W. A., Burstein, S., Rosenfeld, J., Ritzi, E. M. and Watson, D. J. (1973) Prostaglandins 4, 553–565.
[12] Cornette, J. C., Harrison, K. L. and Kirton, K. T. (1974) Prostaglandins 5, 155–164.
[13] Liebig, R., Bernauer, W. and Peskar, B. A., Naunyn-Schmiedebergs Arch. Pharmacol., submitted for publication.
[14] Mathé, A. A. and Levine, L. (1973) Prostaglandins 4, 877–890.

[15] Zusman, R. M. (1972) Prostaglandins 1, 167–168.
[16] Goodriend, T. L., Levine, L. and Fasman, G. D. (1964) Science 144, 1344–1346.
[17] Levine, L. (1967) in: Microcomplement fixation, Handbook of Experimental Immunology (Weier, D. M. ed.) p. 707–719, Oxford, Blackwell.
[18] Anggård, E., Green, K. and Samuelsson, B. (1965) J. Biol. Chem. 240, 1932–1940.
[19] Levine, L., Gutierrex Cernosek, R. M. and Van Vunakis, H. (1972) in: International Conference on Prostaglandins, Vienna (Bergström, S. ed.), p. 71–82, Pergamon Press.