ANTIBODIES AGAINST DEHYDRATION PRODUCTS OF 15-KETO-13,14-DIHYDRO-PROSTAGLANDIN E_2

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1. Introduction

Levels of 15-keto-13,14-dihydro metabolites of prostaglandins (PGs) E_2 and $F_{2\alpha}$ in plasma reflect more accurately PG biosynthesis than levels of the primary PGs [1]. However, so far there are only few reports on the development of radioimmunoassays for the PGE_2 metabolite 15-keto-13,14-dihydro-PGE_2 (KH_2PGE_2) [2–5]. Furthermore, the reliability of these assays seems to be lower than the reliability of radioimmunoassays for the corresponding $PGF_{2\alpha}$ metabolite [5,6]. This is probably a consequence of the chemical instability of KH_2PGE_2, which is easily dehydrated to KH_2PGA_2 [6,7]. KH_2PGA_2 can bind to proteins and, under certain conditions like alkaline pH or in the presence of albumin, rearrange to the stable bicyclic degradation end product 11-deoxy-15-keto-13,14-dihydro-11,16-cyclo-PGE_2 (DKH_2-cyclo-PGE_2) [6–8]. We have described the first radioimmunoassay for KH_2PGE_2 [2]. Now we show that antisera obtained from rabbits immunized with a KH_2PGE_2-conjugate contain not only antibodies against KH_2PGE_2, but also against the KH_2PGE_2 dehydration products KH_2PGA_2 and DKH_2-cyclo-PGE_2. The three antibody populations differ in their specificities as well as in their association constants. With [^{3}H]KH_2PGE_2 or the tritiated dehydration products as tracers the same antisera can be used for radioimmunoassays of KH_2PGE_2, KH_2PGA_2 and DKH_2-cyclo-PGE_2, the specificity and sensitivity of the assays being dependent on the labelled ligand used. The radioimmunoassays for KH_2PGE_2 and KH_2PGA_2 may be used for the direct determination of these compounds under defined conditions. Thus, the radioimmunoassay for KH_2PGA_2 may be especially useful for the determination of the PGE_2 metabolite, when rapid decomposition of KH_2PGE_2 occurs, but is suspended with the formation of KH_2PGA_2 as, e.g., during incubation of KH_2PGE_2 in the absence of albumin at neutral pH and elevated temperatures [7]. Since in the presence of albumin both KH_2PGE_2 and KH_2PGA_2 are not stable and are further transformed to DKH_2-cyclo-PGE_2 [6,7], the radioimmunoassay for DKH_2-cyclo-PGE_2 might be used for the determination of the PGE_2 metabolite in plasma after conversion to the stable dehydration end product as proposed [8]. However, as DHK_2-cyclo-PGE_2 exists in at least three epimerically distinct forms [7], a specificity problem of the radioimmunoassay could emerge at the epimeric level. So far the epimeric ratio formed under various conditions cannot be controlled [7]. Nevertheless, the anti-DKH_2-cyclo-PGE_2 antibodies described here may be especially useful for the radioimmunological determination of KH_2PGE_2 after dehydration in biological material, since these antibodies were produced after immunization with a conjugate synthesized originally with KH_2PGE_2 as hapten and may, therefore, specifically recognize the epimer(s) formed from KH_2PGE_2 under quasi-physiological conditions.

2. Materials and methods

KH_2PGE_2, KH_2PGA_2 and DKH_2-cyclo-PGE_2 as well as other PGs and thromboxane B_2 (TXB_2) were a generous gift of Dr J. Pike, Upjohn Co., Kalamazoo, MI. Their purity was checked by thin layer chromatography in the following solvent systems: chloroform/tetrahydrofuran/acetic acid (100:10:5); ethyl acetate/H_2O/isooctane/acetic acid (110:100:50:20, upper phase); and water-saturated diethyl ether. Only

the latter solvent system separated KH_2PGA_2 from DKH_2-cyclo-PGE_2 sufficiently (R_F values 0.61 and 0.67, respectively). DKH_2-cyclo-PGE_2 was a mixture of at least three epimers (F. A. Fitzpatrick, Upjohn Co., Kalamazoo, personal communication), which could not be separated. [5,6,8,11,14-^{3}H]KH_2PGE_2 (spec. act. 70 Ci/mmol) was obtained from New England Nuclear Co., Dreieichenhain. The labelled ligand for the radioimmunoassay of KH_2PGA_2 was prepared by incubation of 5 μCi [^{3}H]KH_2PGE_2 in 0.5 ml 0.1 M Tris–HCl buffer (pH 8.0) at 37°C for 18 h. After acidification to pH 3.5 the material was extracted into 2 × 4 vol. ethyl acetate and purified by thin-layer chromatography. Similarly, the labelled ligand for the radioimmunoassay of DKH_2-cyclo-PGE_2 was prepared by incubation of 25 μCi [^{3}H]KH_2PGE_2 at 37°C for 18 h in 1 ml human plasma depleted of endogenous PG by charcoal adsorption [9]. The labelled material was extracted and purified as above. The radioactivity co-chromatographing with authentic KH_2PGA_2 or DKH_2-cyclo-PGE_2, respectively, was scraped off and eluted with 5 ml methanol. Possible epimers of the tritiated product co-chromatographing with DKH_2-cyclo-PGE_2 were not separated. The synthesis of an immunogenic KH_2PGE_2-bovine serum albumin conjugate as well as the production and specificity of antisera against KH_2PGE_2 have been described [2]. Binding parameters for antibody populations used under radioimmunoassay conditions were determined according to [10] using the central linear part of Scatchard plots [11]. The plots were corrected for non-specific binding to normal rabbit serum, which was used in the same dilutions as the antisera. Radioimmunoassays were performed as in [9]. Samples were incubated at 4°C overnight and 0.5 ml charcoal suspension (20 mg/ml) was used to separate free and antibody-bound fractions of antigen.

3. Results and discussion

Rabbits immunized with KH_2PGE_2–bovine serum albumin conjugates produced antisera, which not only bound the homologous hapten KH_2PGE_2, but also its degradation products KH_2PGA_2 and DKH_2-cyclo-PGE_2. As albumin promotes the formation of DKH_2-cyclo-PGE_2 from KH_2PGE_2 [6,7], part of the hapten might have already been transformed during preparation of the immunogen. Further dehydration could have occurred after injection of the immunogen. The binding parameters of such an antiserum according to [10] for the three haptens are shown in table 1. The binding constants for KH_2PGE_2 and KH_2PGA_2 are clearly lower than the binding constant for the stable degradation end product DKH_2-cyclo-PGE_2. Binding of KH_2PGE_2 and KH_2PGA_2 to the antiserum is not due to partial conversion of these compounds to DKH_2-cyclo-PGE_2 during the radioimmunoassay incubation. Using thin layer chromatography of labelled compounds after the incubation, formation of DKH_2-cyclo-PGE_2 from either KH_2PGE_2 or KH_2PGA_2 was not detected. However, there was some formation of KH_2PGA_2 from KH_2PGE_2 (~5–10%) during the incubation under the conditions used (4°C, 18 h). The binding parameters given for KH_2PGE_2 may, therefore, be slightly influenced by the instability of this compound during the incubation.

The specificity of the radioimmunoassays for KH_2PGE_2, KH_2PGA_2 and DKH_2-cyclo-PGE_2 using the same antiserum but different tracers is illustrated in table 2. The antibody population used in the radioimmunoassay for KH_2PGE_2 recognizes the homologous hapten and KH_2PGA_2 equally well, while the relative crossreaction of DKH_2-cyclo-PGE_2 is <0.06%. The crossreaction of the anti-KH_2PGE_2 antisera with other PGs and PG metabolites has been

Table 1
Binding parameters according to [11] of antibody populations against KH_2PGE_2, KH_2PGA_2 and 11-deoxy-15-keto-13,14-dihydro-11,16-cyclo-PGE_2

	Final antiserum dilution used in radioimmunoassay	K_a (l/mol)	A_0 (mol/l)
15-keto-13,14-dihydro-PGE_2	1:3000	6.8×10^8	6.0×10^{-10}
15-keto-13,14-dihydro-PGA_2	1:3000	9.7×10^8	4.8×10^{-10}
11-deoxy-15-keto-13,14-dihydro-11,16-cyclo-PGE_2	1:160 000	2.3×10^{10}	0.4×10^{-10}

A_0, concentration of antibody sites; K_a, intrinsic association constant

Table 2
Specificities of anti-PGE_2 metabolite antiserum depending on labelled antigen used

Inhibitor (ng)	50% displacement of labelled ligand in radioimmunoassay for		
	KH_2PGE_2	KH_2PGA_2	11-deoxy-15-keto-13,14-dihydro-11,16-cyclo-PGE_2
KH_2PGE_2	0.6	5.2	65.0
KH_2PGA_2	0.6	0.6	55.0
11-deoxy-15-keto-13,14-dihydro-11,16-cyclo-PGE_2	>1000	>1000	0.04

For final antiserum dilutions used with the tracers see table 1

described [2]. In the KH_2PGA_2 radioimmunoassay KH_2PGA_2 is the most potent inhibitor of binding of label to antiserum (table 2). The relative crossreaction of KH_2PGE_2 is considerable (11.5%), while that of DKH_2-cyclo-PGE_2 is <0.06% (table 2). The cross-reaction of PGA_2 is 1.4% and that of all other compounds tested (PGE_2, PGE_1, $PGF_{2\alpha}$, $PGF_{1\alpha}$, PGD_2, PGD_1, 6-keto-$PGF_{1\alpha}$, 15-keto-PGE_2, 15-keto-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$, TXB_2 and 13,14-dihydro-15-keto-TXB_2) is <0.06%. The radioimmunoassay for DKH_2-cyclo-PGE_2 is highly specific, the relative crossreaction of KH_2PGE_2 and KH_2PGA_2 being only 0.06% and 0.07%, respectively (table 2). The interference of all other PGs and PG metabolites as well as of TXB_2 in this radioimmunoassay is negligible (relative crossreaction <0.01%). Obviously the bicyclic structure of the KH_2PGE_2 degradation product as compared to the typical structure of primary PGs and PG metabolites results in different immunodominant parts of the hapten molecule. In accordance with the higher avidity of the antibodies (table 1) the sensitivity of the radioimmunoassay for DKH_2-cyclo-PGE_2 is considerably higher than that for KH_2PGE_2 and KH_2PGA_2, 40 pg causing 50% inhibition of binding of label to the antiserum (table 2) and the detection limit (10% inhibition of binding of label) being 4 pg.

Our results show, that antibodies can be produced against compounds like KH_2PGE_2 and KH_2PGA_2, which are not completely stable under physiological conditions. However, as expected, the avidity of these antibodies is lower than the avidity of antibodies against the stable degradation product DKH_2-cyclo-PGE_2. The use of stable hapten mimics instead of labile natural compounds for immunization has been suggested to produce antisera of high avidity and specificity [12] and this method might also be suitable for the production of anti-KH_2PGE_2 antisera, which could be used for sensitive radioimmunoassays. The relatively low avidity of the antibodies against KH_2PGE_2 and KH_2PGA_2 may prevent their use for radioimmunological determination of low amounts of the haptens in biological material. Thus, while the sensitivity of our radioimmunoassay for KH_2PGE_2 was sufficient to determine the considerable concentrations of the PGE_2 metabolite found in freshly prepared extracts of human gastric juice [13], it was not possible to detect plasma levels of KH_2PGE_2, which are in the low pg/ml range [14].

A radioimmunoassay for KH_2PGA_2 has so far not been described. This assay may be especially useful for the determination of the PGE_2 metabolite under conditions, where rapid dehydration of KH_2PGE_2 to KH_2PGA_2 takes place, but binding to albumin and/or cyclization to DKH_2-cyclo-PGE_2 does not occur as, e.g., during incubation of KH_2PGE_2 in protein-free solutions at neutral pH and elevated temperatures [7]. Similarly, large amounts of KH_2PGA_2 are formed from KH_2PGE_2 during extraction from aqueous solutions into organic solvents at acid pH [13].

A radioimmunoassay for DKH_2-cyclo-PGE_2 was described [8]. It was suggested that PGE_2 metabolite be measured as DKH_2-cyclo-PGE_2 after complete chemical conversion. Certainly, one can expect a greater reliability of assay results from this method, as, contrary to KH_2PGE_2, DKH_2-cyclo-PGE_2 is stable under various experimental conditions. However, DKH_2-cyclo-PGE_2 exists in at least three epimerically distinct forms [7], the ratio of which formed during various conversion procedures cannot yet be controlled [7]. Valid determination of KH_2PGE_2 as DKH_2-cyclo-PGE_2 requires that the anti-DKH_2-cyclo-PGE_2 antibodies used recognize specifically the epimer(s) of the degradation product formed by the

conversion procedure. The anti-DKH_2-cyclo-PGE_2 antibodies described here were raised by immunization with an immunogen synthesized originally with KH_2PGE_2 as hapten [2]. These antibodies are, therefore, most probably directed against the epimer or mixture of epimers of DKH_2-cyclo-PGE_2 formed from KH_2PGE_2 under quasi-physiological conditions. Consequently, the anti-DKH_2-cyclo-PGE_2 antibodies described here are possibly more suitable for use in radioimmunoassay than antibodies raised by immunization with conjugates of epimeric mixtures of DKH_2-cyclo-PGE_2. Furthermore, the labelled ligand for our radioimmunoassay was also synthesized from [^{3}H]KH_2PGE_2 under quasi-physiological conditions. The possible use of this radioimmunoassay for DKH_2-cyclo-PGE_2 for specific determination of KH_2PGE_2 in biological material after conversion to the stable dehydration product is under investigation.

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