ACTION OF RADIATION INJURY MODIFIERS ON PROSTAGLANDIN E_2 BINDING BY LIVER MEMBRANES OF (CBA × C57BL)F_1 MICE

E. N. Pryanishnikova, Z. I. Zhulanova, and E. F. Romantsev

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The biological action of prostaglandins (PG) has been shown to take place through their binding with specific cell membrane receptors. The nature of the PG receptors is currently being studied. The use of trypsin, pronase, and sulfhydryl reagents (sodium p-chloromercuribenzoate — PCMB, N-ethylmaleimide) has demonstrated a marked decrease in PG binding with specific receptors of the cow corpus luteum [7]. These results indicate that the binding sites of PGE are lipoprotein in nature and contain thiol and sulfhydryl groups. Metal ions, fatty acids, steroids, inhibitors of PG biosynthesis, and sulfur-containing compounds modify PG binding with receptors to different degrees [1, 4, 5]. There are no data in the literature describing the action of radiation injury modifiers on the PG—receptor bond.

The investigation described below was carried out to study the kinetics of PGE_2 binding with mouse liver membrane receptors and the effect of sulfur-containing radioprotectors S-[N-(3-aminopropyl)-2-aminoethyl]thiophosphate (APAETP) and of β-mercaptoethylamine (MEA) on stability of the PGE_2—receptor complex.

EXPERIMENTAL METHOD

The membrane fraction was isolated by the method in [2] from the liver of male (CBA × C57BL)F_1 mice. The purity of the fraction was verified electron-microscopically. Binding of [^{14}C]-PGE_2 with the membrane receptors was analyzed by a modified Rao's method [6]. The incubation mixture contained 0.1 M Tris-HCl, pH 7.3-7.4, 0.25 M sucrose, 1 mM $CaCl_2$, 0.05 μCi [^{14}C]-PGE_2 (from Amersham Corporation, England, 55 mCi/mmole), and 0.1 ml of membrane suspension (protein concentration 16-19 mg/ml). The total volume of the incubation mixture was 0.4 ml.

After incubation at 37°C the tubes were cooled with ice and the volume adjusted to 1 ml with cold Tris-buffer, pH 7.3-7.4. Separation of bound and unbound [^{14}C]-PGE_2 was carried out by centrifugation at 10,000 rpm for 10 min. An aliquot was taken from the supernatant and radioactivity of [^{14}C]-PGE_2 not bound with the membrane fraction was determined on a RackBeta II 1215 counter (from LKB, Sweden). Total radioactivity was determined by adding 0.1 ml Tris-buffer instead of the membrane suspension to the incubation medium. All the remaining operations were carried out as described above. The specific binding was calculated as follows:

$$A_t - A_u = A_b \quad (1)$$

$$A_b = A_{s.b.} + A_{n.b.} \quad (2)$$

$$A_{s.b.} = A_b - A_{n.b.} \quad (3)$$

where A_t denotes total radioactivity; A_u, radioactivity of unbound PGE_2; A_b, total binding; $A_{s.b.}$, specific binding; $A_{n.b.}$, nonspecific binding.

Nonspecific binding was determined in each experiment by incubating with a heat-denatured membrane suspension (5 min, 100°C) or with a thousandfold excess of unlabeled PGE_2.

To block SH groups of membrane proteins, 1 mM PCMB was added to the incubation system. APAETP and MEA were added to the incubation medium in concentrations of 0.38 and 6.6 mM respectively.

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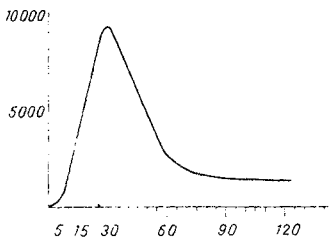

Fig. 1

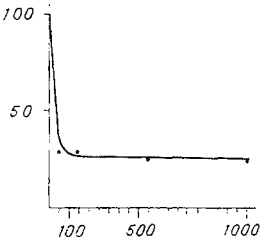

Fig. 2

Fig. 1. Specific binding of [^{14}C]-PGE_2 with mouse liver membranes as a function of incubation time. Abscissa, incubation time (in min); ordinate, radioactivity (in cpm).

Fig. 2. Inhibition of binding of [^{14}C]-PGE_2 with mouse liver membranes by unlabeled PGE_2. Abscissa, concentration of unlabeled PGE_2 (in ng/ml); ordinate, binding (in percent of control).

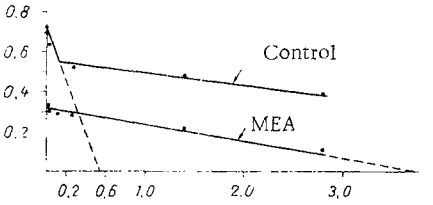

Fig. 3. Scatchard plot for binding of [^{14}C]-PGE_2 with mouse liver membranes. Abscissa, concentration of PGE_2 (in $M \times 10^{-9}$); ordinate, ratio of bound to free [^{14}C]-PGE_2.

EXPERIMENTAL RESULTS

To determine the optimal binding time of [^{14}C]-PGE_2 with the liver cell membranes, incubation was carried out at different time intervals. The results showed that the optimal incubation time was 30 min (Fig. 1). Data on equilibrium binding were obtained in experiments with incubation with increasing concentrations of unlabeled PGE_2 (Fig. 2). These results were subsequently used for a Scatchard plot analysis (Fig. 3), which showed that binding of [^{14}C]-PGE_2 with liver cell membrane receptors is heterogeneous, indicating two different reception sites with the following parameters: 1) a high affinity site with dissociation constant of 0.82×10^{-9} M and with 0.36×10^{9} moles/mg protein binding sites; 2) a low affinity site with dissociation constant of 15.73×10^{-9} M and with 5.31×10^{9} moles/mg protein binding sites.

Data on the effect of PCMB and radiation injury modifiers (APAETP and MEA) on binding of PGE_2 with liver cell membrane receptors are given in Table 1. Preincubation of the membranes with PCMB followed by addition of [^{14}C]-PGE_2 leads to a reduction in binding. In cases when PCMB was added to the medium before incubation of the membranes with [^{14}C]-PGE_2, inhibition of binding was more marked. These results suggest that SH-groups are present in the composition of the PGE_2 receptor and that occupation of the membrane PGE_2 receptors affords partial protection against the action of compounds reacting with the SH-group of membrane proteins [4].

To assess the role of radiation injury modifiers in the mechanism of PGE_2 membrane reception experiments were carried out with preincubation of radioprotectors with membranes followed by the addition of [^{14}C]-PGE_2, and also with preincubation of [^{14}C]-PGE_2 with membranes followed by the addition of APAETP and MEA.

On preincubation of the membranes with APAETP and MEA (Table 1) binding of [^{14}C]-PGE_2 with the membrane receptors was inhibited, possibly as a result of disturbance of the conformation of the membrane receptor proteins as a result of rupture of disulfide cross-linkages [8]. These compounds can also induce a reduction in binding through their interaction with free disulfide and sulfhydryl groups of membrane lipoproteins.

TABLE 1. Effect of PCMB and Radiation Injury Modifiers on Binding of [^{14}C]-PGE_2 with Liver Cell Membrane E_2 Receptors

Compound	Preincubation with test compound	Preincubation with [^{14}C]-PGE_2
	percent inhibition	
PCMB	72,64	43,63
APAETP	50,44	76,72
MEA	45,92	61,57

It is also stated in the literature [3] that sulfur-containing substances interact directly with PG. In the same way MEA and APAETP can form covalent bonds with PGE_2 molecules, but the newly formed compounds will no longer bind with receptors for PG.

On preincubation of the membranes with [^{14}C]-PGE_2 followed by addition of radioprotectors, a decrease in binding also was observed (Table 1). Partial "loss" of binding under these conditions may reflect conformational changes in macromolecules of the binding site under the influence of APAETP and MEA, resulting in fairly considerable dissociation of the PGE_2 — receptor complex. Furthermore, as was shown previously, PGE_2 protects membrane proteins against the action of compounds reacting with SH and S — S groups of membrane proteins.

On the basis of data in the literature on the character of changes in the kinetics of receptor binding with PGE_2 under the influence of compounds reacting with S — S and SH groups of cell membranes [7] and our own suggestions, experiments were carried out to study competitive inhibition of binding with one of the radioprotectors, namely MEA, using the Scatchard plot technique (Fig. 3). Under these circumstances the following changes in binding kinetics were observed: 1) absence of the high-affinity site; 2) the calculated dissociation constant ($K_d = 12.58 \times 10^{-9}$ M) was practically identical with K_d for the low-affinity site in the control; 3) the number of binding sites ($N = 2.43 \times 10^9$ moles/mg protein) was reduced by 2.19 times compared with the control value for the low-affinity site.

The results thus indicate that sulfur-containing radioprotectors are actively involved in the binding of PG with mouse liver cell membranes. It can be postulated on the basis of these results that a similar mechanism will also be involved when the radiation injury modifiers studied in these experiments are administered *in vivo*.

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