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Interactions of Malondialdehyde and 4-Hydroxynonenal with Rat Liver Plasma Membranes and their Effect on Binding of Prostaglandin E_2 by Specific Receptors

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We investigated effect of aldehydic products of lipid peroxidation, malondialdehyde (MDA) and 4-hydroxynonenal (HNE) on prostaglandin (PG) E_2 receptors of liver plasma membranes. The modification of the membranes by MDA diminished PGE_2 binding, decreasing receptor affinity for PGE_2 and receptor density whereas HNE increased PGE_2 binding, enhanced receptor density but did not changed receptor affinity. ESR study showed the decrease of the whole membrane fluidity after modification by MDA whereas HNE lowered membrane fluidity only in the internal zone of lipid bilayer and increased it in the surface area. The possible effects of membrane changes caused by MDA and HNE on PGE_2 receptor parameters are discussed.

Keywords: Malondialdehyde, 4-hydroxynonenal, prostaglandin receptors, plasma membranes

Abbreviations: HNE, 4-*trans*-hydroxynonenal; K_{ass}, constant of association; MDA, malondialdehyde; PGE, prostaglandin E

INTRODUCTION

Malondialdehyde (MDA) and 4-hydroxynonenal (HNE) are most actively investigated aldehydic end-products of lipid peroxidation.[1] Being highly reactive compounds, MDA and HNE interact with lipid and protein compounds of living mamalian cells, leading to cell damage. Hepatocytes are target cells for endogenously produced aldehydes. Acetaldehyde formed from ethanol during alcohol intoxication favours a neutral lipids accumulation into hepatocytes as an initial step of alcoholic liver injury.[2] Liver damage in diabetes is mediated through glycation of liver proteins by an excess of aldehydic form of glucose.[3] MDA and HNE are also hepatotoxic aldehyde[1,4] changing metabolic parameters in the liver. The intimate mechanisms of these alterations can be connected with distur-

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bances in the signal transduction into hepatocytes depending on the cAMP formation[5] or mediated by protein kinase C.[6]

Prostaglandins (PG) of the E series have a wide variety of biological activities in different tissues. Usually the cellular mechanisms of PGE effects are associated with alterations in adenylate cyclase activity and, in turn, cAMP level through specific PG receptors.[7] PGE have hepatoprotective properties preventing liver damage in chronic alcohol intoxication[8] and after carbone tetrachloride treatment.[9] Earlier we demonstrated that the alkylation of liver plasma membranes by acetaldehyde decreases the PGE receptors density, competing with carbonyl-containing PGE for binding sites on the receptor.[10] The present studies were designed to examine high-specific binding of PGE to receptors of liver plasma membranes in the presence or absence of either MDA or HNE. Furthermore, the effect of these aldehydes on the state of lipid bilayer of the liver plasma membrane and anular membrane lipids was studied.

MATERIALS AND METHODS

[^{3}H]PGE_2 (140 Ci/mmol) was purchased from the Radiochemical Center (Amersham, England) and was chromatographed upon arrival against authentic PG standard. All the spin probes were obtained from "Sigma" (USA). Unlabelled PGE_2 was obtained from "Kemasol", (Tallinn, Estonia). MDA was prepared from 1,1,3,3,-tetramethoxypropane ("Aldrich", USA) by acid hydrolysis.[1] HNE was a generous gift of Prof. H. Esterbauer (University of Graz, Austria).

Male rats of the Rappolovo colony weighing approximately 250 g were used for liver plasma membrane preparation. The animals were decapitated after the liver perfusion in situ with 1 mM sodium bicarbonate. Plasma membranes were isolated from whole liver homogenates using the method of Pospelova[11] as described elsewhere.[10] Protein concentration was determined by the method of Lowry *et al.*[12] The PGE binding studies were carried out by the use of system containing 50 mM Tris-HCl buffer, pH 7,5, 1 mM $MgSO_4$ and 12 nM of [^{3}H]-PGE_2. The total volume of the incubation medium was 350 µl. The binding was initiated by the addition of 50 µl of the membrane suspension containing 165 µg of protein. The samples were incubated at 5°C in a shaking water bath for 30 min. The binding was terminated by placing the tubes into an ice bath. Membrane-bound [^{3}H]PGE_2 were precipitated by centrifugation in a microfuge for 15 min at 2200 g and at 40°C. Each estimation was an average of three experiments. The pellet was washed once with 1,0 ml of 50 mM Tris-HCl buffer, pH 7,5, containing 1 mM $MgSO_4$, resuspended in 1 ml of this buffer and transferred to a scintillation vial. The pellet and supernatant samples were counted in a Mark II liquid scintillation counter (Nuclear Chicago, USA) using an universal cocktail.

The binding data were transformed and plotted according to Scatchard.[13] To investigate the binding parameters [^{3}H]PGE_2 was used in the concentration ranges from $25 \cdot 10^{-10}$ M to $25 \cdot 10^{-9}$ M. The competition for [^{3}H]PGE_2 binding sites was performed under the same conditions with unlabelled PGE_2 added in an 1- to 1000-fold excess. The time course and reversibility of the [^{3}H]PGE_2 binding were studied using membranes incubation during 60 min. To some reactions tubes 1000-fold excess of unlabelled PGE_2 was added after 30 min of incubation. Liver plasma membranes were modified by MDA or HNE (10^{-5} M) in 50 mM Tris-HCl buffer, pH 8,0 at 22°C for 4 h.

All the ESR spectra were taken at room temperature (22°C) on ERS-220 spectrometer (Germany). The usual instrumental parameters were: microwave power 5 mW; center of the field 3300 G; sean range 100 G. Quartz tubes of 1mm internal diameter were used. Doxyl derivatives of stearic acid with nitroxyl radical in 5- and 12-positions (5-DSA and 12-DSA, respectively) were used for a scanning of liver plasma membranes. The period of rotatory diffusion (τ) was calculated using a routine method.[14]

RESULTS

Figure 1A shows the kinetics of [^{3}H]PGE_2 binding by liver plasma membranes. The specific binding increased in a time-dependent manner. An apparent equilibrium occured between 20–30 min, after which the maximal binding was retained for further 30 min. The addition of 1000-fold excess of unlabelled PGE_2 at the 30 min time point displaced the labelled ligand by nearly 80%. The preincubation of liver plasma membranes with MDA resulted in a significant decrease of the [^{3}H]PGE_2 binding and membrane modification by HNE increased the amount of bounded [^{3}H]-

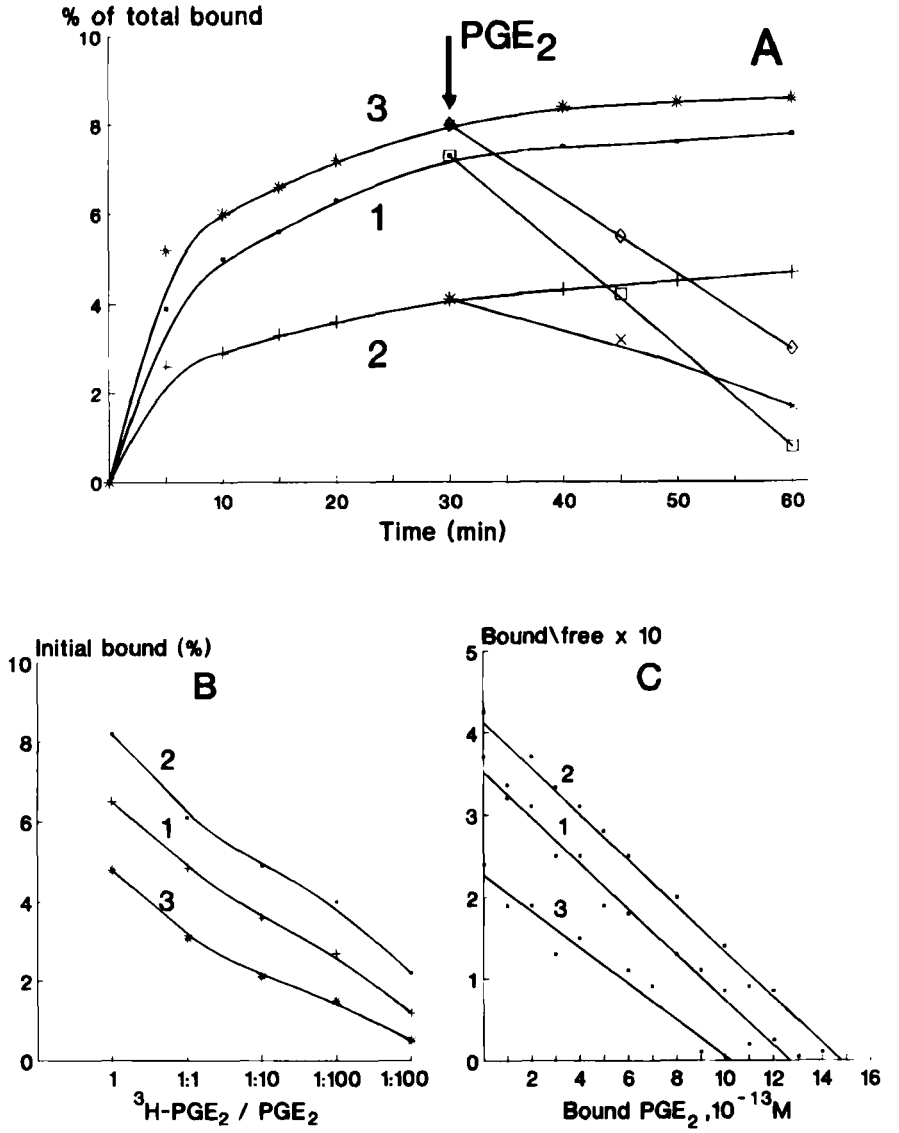

FIGURE 1 Effect of MDA and HNE on binding parameters of PGE_2 to the liver plasma membranes: A—Time course of binding of [^{3}H]-PGE_2 to the liver plasma membranes and the dissociation of the ligand by an excess of unlabelled PGE_2; B—Effect of unlabelled PGE_2 on binding of the [^{3}H]-PGE_2 by native and modified by both MDA and HNE liver plasma membranes; C—Scatchard analysis of [^{3}H]-PGE_2 binding to native and both MDA and HNE modified liver plasma membranes. Designations: 1—native membranes; 2—membranes modified by MDA; 3—membranes modified by HNE.

PGE_2. In both the cases, the displacement of $[^3H]PGE_2$ from the binding sites on modified membranes by the unlabeled PGE_2 excess was less pronounced than that on native membranes.

Figure 1B represents the results of the membrane incubation in the presence of increasing amounts of unlabelled PGE_2 with a constant amount of $[^3H]PGE_2$. The binding of $[^3H]PGE_2$ was inhibited by unlabelled PGE_2 in a dose-dependent manner which seems to suggest specificity of the PGE_2 receptor. The total binding of $[^3H]PGE_2$ to liver plasma membranes was significantly less pronounced in MDA-modified membranes than in native membranes, whereas the nonspecific binding was approximately the same in both the modified and native membranes. The modification of membranes by HNE only slightly enhanced the total binding of $[^3H]PGE_2$ to receptors, but dramatically increased the nonspecific binding.

The Scatchard analysis of the binding data gave a straight line indicating the existence of a single class of high affinity binding sites (Fig. 1C). The data on the equilibrium association constant (K_{ass}) and the density of receptors (fmol/mg proteins) are summarized in Table 1. The modification of liver plasma membranes by MDA led to a decrease of the K_{ass} for $[^3H]PGE_2$ and slightly reduced the PGE_2 receptor density. The alkylation of membranes by HNE enhanced the receptor density, but did not essentially changed the K_{ass}.

The effect of MDA and HNE on the structural prolife of liver plasma membranes was investigated by ESR spectrometry using spin probes with different depths of nitroxyl label immersion into membranes. The MDA concentrations up to 0.15 mM did not change the behavior of 5-DSA whose nitroxyl radical was located in the surface zone of membranes (Fig. 2). The elevated MDA concentration elongated the rotatory correlation period (τ) of the spin probe. The alkylation of membranes with HNE (the initial concentration was 0.15 mM) diminished the value of this parameter for 5-DSA. MDA and HNE changed unidirectionally the rotatory correlation period for the spin probe, 12-DSA, with the nitroxyl radical being located in deeper areas of the lipid bilayer. However, the MDA modification of membranes raised the value of this parameter to a greater extent than did the incubation with HNE.

DISCUSSION

The effective displacement of $[^3H]PGE_2$ by the excess of unlabelled PGE_2 provides evidence for high specificity of the labelled ligand binding sites on the membrane. This is confirmed by the higher K_{ass} values for PGE, which agrees with our earlier data[10] and indicates an interaction of a receptor-ligand type. The MDA treatment of membranes essentially disturbed the specific binding of $[^3H]PGE_2$, decreasing the density of the binding sites. This may be related to a direct MDA modification of the receptor functional groups responsible for the PGE_2 binding, as well as to a decrease of the K_{ass} value, which is probably due to the alterations in the receptor molecule conformation induced by changed properties of its lipid surroundings.

At the same time the HNE modification of membranes increased the amount of membrane-bound $[^3H]PGE_2$, which might be a con-

TABLE I The effect of liver plasma membranes modification by MDA and HNE on $[^3H]$-PGE_2 binding parameters

Binding Parameters	Native Membranes	Membranes Modified by MDA	Membranes Modified by HNE
K_{ass}, M^{-1}	$0.4 \cdot 10^9$	$0.25 \cdot 10^9$	$0.42 \cdot 10^9$
Receptor density, fmol/mg of protein	$110 \cdot 10^{-13}$	$90 \cdot 10^{-13}$	$160 \cdot 10^{-13}$

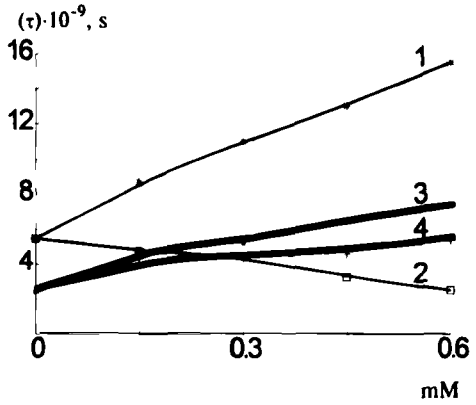

FIGURE 2 Effect of MDA and HNE on the rotatory correlation period (t) of 5-doxylstearic acid and 12-doxylstearic acid bounded to liver plasma membranes. Designations: 5-DSA, membranes modified by MDA (1) and HNE (2); 12-DSA, membranes modified by MDA (3) and HNE (4).

sequence of an increased number of PGE_2 binding sites. As Figure 1 and 2 demonstrate, this was mainly related to nonspecific [^{3}H]PGE_2 binding. The spin label behaviour indicated that the modification of liver plasma membranes by the aldehydes investigated changed the membrane microviscosity. The rigidity of the internal membrane zone, as assessed by the 12-DSA behaviour, was enhanced under the influence of both MDA and HNE, the MDA effect being more pronounced. However, the microviscosity of the surface zone was increased following the MDA modification and diminished after the HNE alkylation.

It is known that MDA is capable of modifying protein sulfhydryl and amino groups, producing mainly protein-protein cross-links.[15,16] The formation of similar cross-links leads to a more rigid membrane structure, which is confirmed by the increased rigidity of both the inner and surface membrane areas. We assume that a similar modification of the membrane alter the conformation of membrane proteins including PGE_2 receptor which changes functional properties of this protein. The order of membrane lipid bilayer is significantly increased by HNE in the presence of 5-DSA[17] that indicate on relaxation of protein-lipid contacts in membrane. A consequence of these aldehyde-induced changes may be disturbances in the PGE_2 binding to receptors.

Earlier we have shown the feasibility of acetaldehyde modification of the ε-amino groups of PGE_2 receptor lysine residues, which decreased the number of binding sites, but unaffected the K_{ass} value.[10] Therefore we cannot also rule out a direct modification of receptor protein functional groups by MDA and HNE.

If the MDA effect on the interactions of PGE_2 with receptor can be interpreted in terms of the enhanced membrane rigidity, then the effect of HNE on the PGE_2 binding by liver plasma membranes which is opposite to the MDA effect is associated to decreased membrane microviscosity. It is evident that the dissolving of the 4-HNE hydrofobic molecules in the lipid bilayer induced fluidization of the surface area of liver plasma membrane which correspond to the depth of the HNE molecula immersion into membrane. As we shown earlier, similarly decreased microviscosity of the surface zone of red cell membrane was observed following its modification by aliphatic aldehydes.[18] The decreased microviscosity of the surface zone of liver plasma membrane favoured an increase in the [^{3}H]PGE_2 binding due to nonspecific binding.

In conclusion, the changes in the functional parameters of the [^{3}H]PGE_2 binding by the liver plasma membranes found following the modification by lipid peroxidation end-products were related to the structural alteration in the membrane characterized by changes in the profile of its rigidity. In its turn, this depends on the aldehyde properties, particularly on hydrophobicity, and is governed by the aldehyde localization in the membrane.

Acknowledgments

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