

Nonenzymatically evoked and cytochrome P450-dependent lipid peroxidation inhibits synthesis of phosphatidylethanolamine via the ethanolamine base exchange reaction in rat liver microsomes

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Abstract In the present study the relationship between lipid peroxidation, changes in the redox state of membrane and phosphatidylethanolamine (PE) synthesis via base exchange reaction in rat liver microsomes was investigated. It was found that PE synthesis is enhanced in the presence of antioxidants, butylated hydroxytoluene (BHT), or unsaturated free fatty acids. Prooxidants, *tert*-butyl hydroperoxide (BHP), ferrous ions combined with ascorbate or NADPH (via cytochrome P450-dependent proteins), increased the amount of lipid peroxidation products in the membrane, and in consequence inhibited the reaction. The effect of BHP was fully reversed by reduced glutathione and dithiothreitol (DTT), whereas the effect of other compounds could be reversed only by BHT. In contrast, a reversal of the inhibitory effect of cadmium ions on base exchange activity was observed in the presence of DTT, but not BHT. Therefore, both the -SH/-S-S- ratio in the membrane, affected by BHP and cadmium ions, and the lipid hydroperoxides (rather than aldehydes), generated by ferrous ions and ascorbate or NADPH, are equally responsible for the inactivation of the ethanolamine base exchange enzyme in rat liver microsomes. This may suggest that the synthesis of PE via the base exchange reaction may be considered an element of the superfine cellular machinery involved in the repair of damage to unsaturated fatty acid chains of phospholipids caused by reactive oxygen species under oxidative stress.

Key words: Phospholipid base exchange reaction; Phosphatidylethanolamine; Lipid peroxidation; Antioxidants; Cytochrome P450; Rat liver

1. Introduction

Phosphatidylethanolamine (PE) is one of the major class of phospholipids in eukaryotic cells, where it constitutes 20–40% of the total phospholipid content of various membranes. Despite *de novo* synthesis and decarboxylation of phosphatidylserine (PS) [1], PE is formed from phosphatidylcholine (PC) or PS by a phospholipid base exchange (BE) reaction, specific to ethanolamine [2]. However, in mammalian cells BE is the

sole pathway for PS formation. Sundler et al. [3] reported that in isolated rat hepatocytes, at physiological concentrations of ethanolamine (25 μ M), 8–9% of the total PE synthesis could be attributed to Ca^{2+} -dependent incorporation of ethanolamine via the BE reaction. The reaction does not result in a net increase of the total PE content, but is rather responsible for significant remodelling of preexisting membrane phospholipids. The most abundant molecular species of PS and PE are known to contain long-chain polyunsaturated fatty acids: arachidonic (20:4), docosatetraenoic (22:4) and docosahexaenoic (22:6) in the *sn*-2 position of their glycerol moiety [4]. Furthermore, Ellingson and Seenaiah [5] have documented that stearyl-polyunsaturated molecular species of PE and PC are preferentially converted to PS by the phospholipid BE reaction in rat liver microsomes.

PE, kept in a bilayer configuration by interactions with other membrane phospholipid molecules, is able to induce local nonbilayer structures by creating hexagonal phases [6]. This property of PE may be responsible for regulation of the activity of many membranous enzymes, for example cytochrome P450 [7]. The latter class of enzymes participates in hydroxylation processes of different hydrophobic molecules, like fatty and bile acids, phospholipids, steroid hormones and/or xenobiotics [8]. Moreover, the PE hexagonal phase configuration was found to be highly sensitive to peroxidation processes [9], by which the unsaturated fatty acid moieties of phospholipid molecules are converted into fatty acid hydroperoxides, other lipid peroxidation intermediates, and finally to malondialdehyde (MDA). The latter molecule has the capacity to bind to the amino groups of phospholipids and proteins [10], while various lipid peroxidation intermediates, by interacting with the evolutionarily conserved redox amino acid sequence (-Cys-Gly-Pro-Cys-) of peptides [11], may influence enzymatic and transport activities of several integral ER membrane proteins such as thioredoxin [11,12], and protein disulfide isomerase [13].

The present study was undertaken to obtain more detailed information on the mechanism of modulation of the ethanolamine BE activity by the amount of lipid peroxides in membrane (both that created nonenzymatically and through the cytochrome P450-dependent pathway), and by the redox state of ER membranes.

2. Materials and methods

2.1. Chemicals

Ethanol-1-ol-2-amine hydrochloride, HEPES, MOPS, glucose-6-phosphate (monosodium), *tert*-butyl hydroperoxide (BHP), butylated hydroxytoluene (BHT), 1,1,3,3-tetraethoxypropane (malonylaldehyde bis [diethylacetal]), and fatty acids (sodium salts) were obtained from

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Abbreviations: PS, phosphatidylserine; PE, phosphatidylethanolamine; ER, endoplasmic reticulum; BE, base exchange; G-6-Pase, glucose 6-phosphatase; DTT, dithiothreitol; BHT, 3,5-di-*tert*-butyl-4-hydroxytoluene; BHP, *tert*-butyl hydroperoxide; pCMB, *p*-chloromercuribenzoic acid; NEM, *N*-ethylmaleimide; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde

In memory of Professor Józef Zborowski, who passed away on 23 December 1993.

Sigma (USA). [2-¹⁴C]Ethan-1-ol-2-amine (54 mCi/mmol) was purchased from Amersham (UK). D-[U-¹⁴C]Glucose-6-phosphate (300 mCi/mmol) was from ARC Inc. (USA). Among -SH group modifying reagents, *N*-ethylmaleimide (NEM) was supplied by Merck (Germany), and dithiothreitol (DTT), 2-mercaptoethanol, mersalyl and *p*-chloromercuribenzoic acid (pCMB) by Sigma (USA). All other chemicals were of the highest purity commercially available.

2.2. Microsomes

Adult male (or female, as stated in the text) Wistar rats weighing 150–180 g were used throughout the study. In most cases, the animals were killed after being starved for 16 h. Liver ER membranes were prepared according to Rakowska et al. [14], resuspended at a protein concentration of 10–20 mg/ml in a buffer containing 250 mM sucrose, 40 mM HEPES, pH 7.4, and stored up to 2 months at –70°C.

2.3. Measurements of phospholipid base exchange activity

The reaction mixture for measurements of BE activity contained 0.25 mg of microsomal protein in 0.25 ml of 40 mM HEPES, pH 7.4, 1 mM CaCl₂, and 50 μM [2-¹⁴C]ethanolamine (spec. act. 1.5 mCi/mmol) or as otherwise indicated. The incubation was carried out at 37°C in a water bath shaker as long as the BE activity remained linear, i.e. up to 20 min. The reaction was terminated by the addition of 2.0 ml of chloroform:methanol mixture (1:2, v/v). Lipids were extracted and incorporation of radiolabelled substrate into PE assessed by scintillation counting in a Beckman LS 6000TA counter (USA). In each assay the nonincubated blank samples (immediately after addition of substrate) were used to measure nonspecific binding of ethanolamine to microsomes (5–10% of total lipid-associated radioactivity). The results obtained were subsequently subtracted from the respective experimental data.

2.4. Lipid peroxidation

Nonenzymatic lipid peroxidation was evoked by preincubation of liver microsomes (2 mg protein/ml) with FeSO₄/ascorbic acid or with other oxidizing agents, as indicated in the legends to figures and in the text, in 40 mM HEPES (pH 7.4) buffer for 0–10 min at 37°C. As a control, microsomes were treated in a similar manner without the oxidizing agent. The reaction was terminated by the addition of BHT to a final concentration of 0.1 mM, followed by determination of ethanolamine BE activity. In a parallel sample, the extent of lipid peroxidation was measured as the level of thiobarbituric acid reactive substances (referred to throughout this paper as TBARS), according to Uchiyama and Mihara [15]. MDA, obtained by acetic hydrolysis of tetraethoxypropane, was used as a standard. NADPH-dependent lipid peroxidation was achieved in the presence or absence of ferric cations, as described elsewhere [16,17].

2.5. Other procedures

Protein concentration was determined according to Lowry et al. [18] or Bradford [19] with bovine serum albumin as a standard. Phospholipids were extracted from membranes according to Bligh and Dyer [20] and quantified by inorganic phosphorus measurements, as described by Rouser et al. [21]. The number of -SH groups in rat liver microsomes was determined spectrophotometrically at 412 nm, by Ellman reaction [22], using $\epsilon = 13\,600\text{ M}^{-1} \times \text{cm}^{-1}$ for calculations. Glucose-6-phosphatase (G-6-Pase) activity was assayed in a buffer consisting of 50 mM MOPS, pH 6.5, and 2 mM EGTA, by radiometric determination of [U-¹⁴C]glucose hydrolysed from 0.5 mM [U-¹⁴C]glucose-6-phosphate (0.16 mCi/mmol), according to Kitcher et al. [23].

3. Results and discussion

3.1. Alterations of ethanolamine base exchange activity in the presence of fatty acids

Our previous experiments revealed that serine BE activity is regulated by the phospholipid pattern of liver microsomal membranes [14]. The same conclusion has been reached by Vecchini et al. [24] for the ethanolamine BE reaction in sarcolemma membranes isolated from cardiomyopathic hamster. It has also been reported that in rat brain microsomes, the reaction is modulated by unsaturated long-chain fatty acids

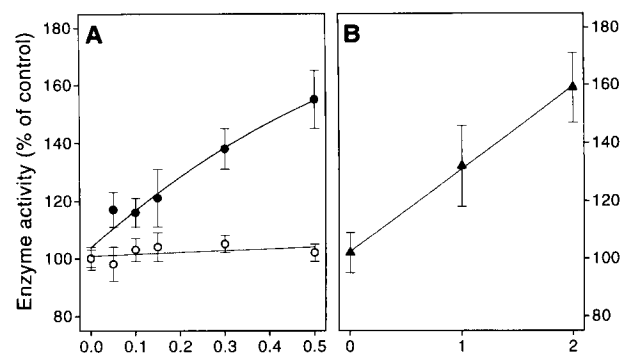


Fig. 1. Alterations of ethanolamine base exchange activity by fatty acids. (A) Rat liver microsomes were preincubated with oleic (●) or stearic (○) acid at the concentrations shown indicated, for 10 min at room temperature, followed by the assay of enzymatic activity for 20 min at 37°C, as described in Section 2. (B) The enzyme activity in the presence of stearic (18:0), oleic (18:1) or linoleic (18:2) fatty acid (final concentration 0.3 mM) is plotted versus the number of double bonds in a fatty acid molecule. Mean results of three experiments \pm S.D. are shown.

[25]. To study the effect of the latter on ethanolamine incorporation into phospholipids of rat liver microsomal membranes, various free fatty acids at a final concentration not exceeding 0.5 mM were used. Under these conditions, microsomal membranes were not solubilized, as tested by turbidity measurements at 540 nm (not shown). We found that oleic acid, but not the saturated fatty acids, palmitic and stearic acid, stimulated the ethanolamine BE activity in a concentration-dependent manner (Fig. 1A). Moreover, a positive correlation was found to exist between the stimulatory effect of an exogenous fatty acid and the number of double bonds in its molecule (Fig. 1B). Analysis of kinetic parameters of the ethanolamine BE reaction assayed in the presence of 0.3 mM fatty acid and 1 mM CaCl₂ showed that V_{\max} values were not significantly affected, whereas K_m for ethanolamine decreased to one-fourth and one-third in the presence of oleic (18:1) and linoleic acid (18:2), respectively, as compared to control values (no fatty acid or stearic acid added). It can be speculated that unsaturated fatty acids stimulate BE activity via enhancement of either membrane bilayer fluidity [26] or phospholipid substrate accessibility for the enzyme. An increase in rotational motion of enzymatic protein and in phospholipid transbilayer movement cannot be excluded, either.

It is worth noting that, in our experimental system, oleic acid might act as a potent antioxidant, since it has been found to inhibit lipid peroxidation in rat intestinal mucosa [27]. In addition, a large difference in the rate of PE synthesis via the BE reaction has been observed between the liver microsomes isolated from male ($36.9 \pm 0.9\text{ pmol} \times \text{min}^{-1} \times \text{mg}^{-1}\text{ protein}$, $n=4$) and female ($96.8 \pm 4.5\text{ pmol} \times \text{min}^{-1} \times \text{mg}^{-1}\text{ protein}$, $n=2$) rats fed ad libitum. This prompted us to consider the up-regulation of the ethanolamine BE activity by estrogens, acting as natural antioxidants [28].

To test the hypothesis on the protective effect of antioxidants on the ethanolamine BE activity, the reaction medium was supplemented with BHT. Indeed, a six-fold increase (from $13.0 \pm 2.8\text{ pmol} \times \text{min}^{-1} \times \text{mg}^{-1}\text{ protein}$ to 81.6 ± 4.0) in ethanolamine incorporation into membrane phospholipids has been observed in the presence of the hydrophobic radical scavenger when the base exchange reaction was carried out in the

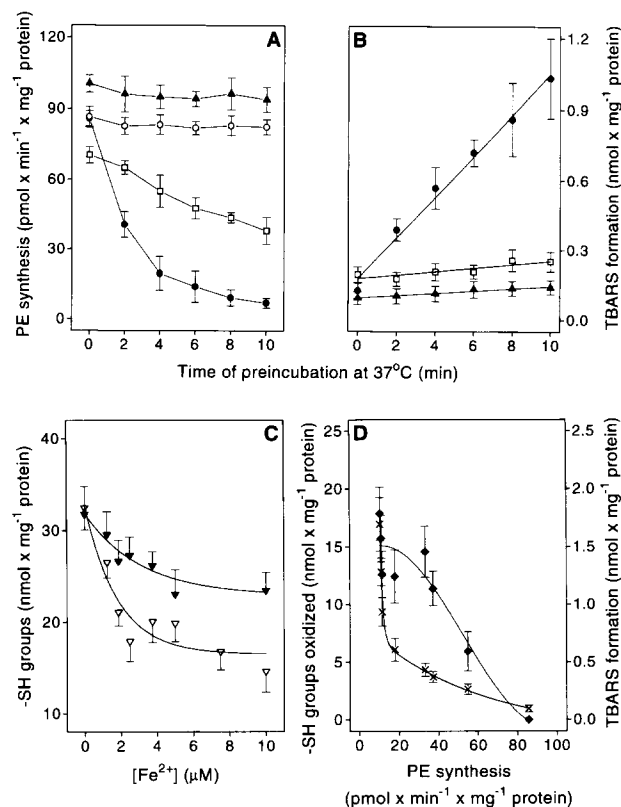


Fig. 2. Effect of lipid peroxidation in the presence of Fe^{2+} /ascorbate on synthesis of PE via the base exchange reaction. Rat liver microsomes (1 mg/ml) were preincubated at 37°C with no effectors (○) or in the presence of 5 μM FeSO_4 and 100 mM ascorbic acid, and no further additions (●) or plus 0.1 mM BHT (△) or plus 1 mM DTT (□). The same symbols are used for A and B. At the time indicated, the preincubation was terminated by the addition of BHT to 0.1 mM concentration (except for the control sample preincubated without Fe^{2+} /ascorbate and the system already containing BHT), followed by the assay of ethanolamine base exchange activity for 20 min at 37°C (A) or determination of thiobarbituric acid reactive substances (TBARS) (B), as described in Section 2. (C) Determination of the number of -SH groups by Ellman reaction in microsomes incubated for 10 min at 37°C with increasing concentrations of FeSO_4 , at a constant 20-fold excess of ascorbic acid, in the absence (▽) or presence (▼) of 0.1 mM BHT. (D) Correlation between the ethanolamine base exchange activity diminution and TBARS formation (×) or oxidation of -SH groups (♦) in microsomes preincubated as described in the legend to C. Mean values \pm S.D. of 3–5 experiments are shown.

presence of low Ca^{2+} concentrations (50 μM). In the presence of 1 mM Ca^{2+} this effect was not so pronounced.

On the other hand, the well-known prooxidant BHP [29], used at a millimolar concentration, inhibited the reaction with IC_{50} of 0.6 mM, and stimulated the thiobarbituric acid-reactive compounds formation (not shown). A hydrophilic antioxidant, 5 mM reduced glutathione (GSH), and 1 mM DTT, protected the reaction against BHP (not shown), while 0.1 mM BHT shifted the IC_{50} for BHP to higher concentrations (2.7 mM). Instead of causing, as expected, peroxidation of phospholipids, BHP was responsible for decreasing the -SH/-S-S- ratio in microsomal membranes [29]. The -SH/-S-S- equilibrium is a well recognized factor influencing the activity of such ER enzymes of physiological importance as G-6-Pase [30,31], cytochrome P450, and aldehyde dehydrogenase [30]. In addition, the molecular mechanism of oxidative stress, strongly affecting the redox state within a cell, is frequently ascribed to destabilization of the membrane lipid bilayer, appearance of hexagonal lipid structures, separation of lipid molecules in the lateral plane of the membrane (formation of lipid microdomains), and changes in membrane permeability to cations and solutes [29].

3.2. Nonenzymatic lipid peroxidation and ethanolamine BE activity

Preincubation of rat liver microsomes at 37°C for 0–10 min with Fe^{2+} or Fe^{2+} /ascorbate (endogenous Ca^{2+} concentration not exceeding 0.1 μM) caused exponential diminution of PE synthesis via the BE reaction (Fig. 2A), as well as inhibition of hydrolysis of glucose-6-phosphate (from 68.5 to 11.1 nmol of glucose \times min⁻¹ \times mg⁻¹ protein), catalysed by ER G-6-Pase. This effect reached a maximum at 5 μM Fe^{2+} , and was accompanied by a linear enhancement of TBARS production (Fig. 2B). No such inhibition was observed in the presence of 10–100 μM Fe^{3+} (in the form of either chloride or sulfate). It is worth noting that Fe^{2+} did not compete with calcium ions in the course of the BE reaction. The K_m for Ca^{2+} was 0.65 ± 0.15 mM in the absence, and 0.71 ± 0.13 mM in the presence of 10 μM Fe^{2+} .

Fe^{2+} /ascorbate is also a potent oxidizing system of membrane monothiol groups [30]. In Fig. 2C the influence of increasing concentrations of the oxidizing agent on the number of -SH groups is shown. Supplementation of the preincubation medium with 0.1 mM BHT provided only a moderate protection against -SH group oxidation, however, inhibition of PE synthesis by Fe^{2+} , by ascorbic acid or by Fe^{2+} /ascor-

Table 1
Effect of the -SH group modifying reagents and prooxidants on the ethanolamine base exchange activity in rat liver microsomes

| Effector | Concentration | Enzyme activity ^a (% of control) | TBARS formation ^b (pmol \times mg ⁻¹ protein) |
|-----------------------------|-------------------------|---|---|
| Mersalyl | 2 μM | 100 | 99.4 |
| CMB | 50 μM | 63 | 118.3 |
| NEM | 1 mM | 66 | 94.5 |
| 2-Mercaptoethanol | 1 mM | 100 | 99.4 |
| Cd^{2+} | 25 μM | 39 | 80.4 |
| DTT | 1 mM | 73 | 245.8 |
| Fe^{2+} | 5 μM | 72 | 105.7 |
| Ascorbate | 0.1 mM | 82 | 174.9 |
| Fe^{2+} /ascorbate | 5 μM /0.1 mM | 12 | 1053.9 |

Microsomes were preincubated for 10 min at room temperature with mersalyl, pCMB, NEM, or 2-mercaptoethanol, and at 37°C with DTT, FeSO_4 and/or ascorbate. CdCl_2 was added directly to the assay medium.

^aControl enzyme activity was 88.7 ± 4.5 pmol \times min⁻¹ \times mg⁻¹ protein ($n=10$).

^bTBARS level in microsomes with no additions amounted to 93.1 ± 15.6 pmol \times mg⁻¹ protein ($n=7$).

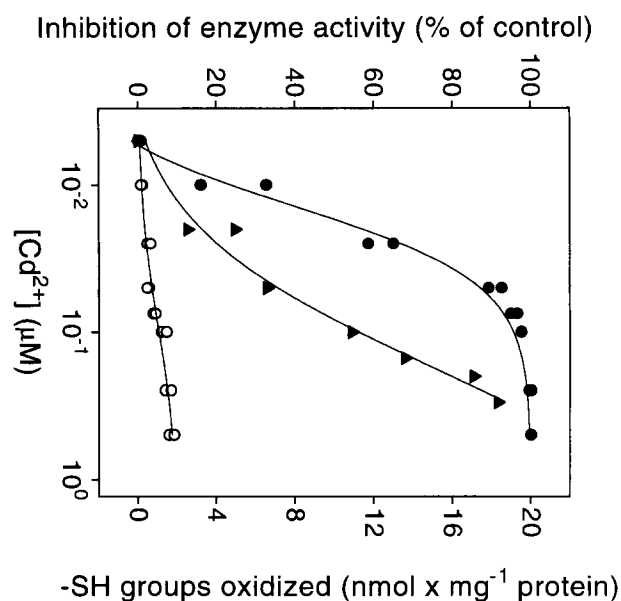


Fig. 3. Effect of Cd^{2+} on the ethanolamine base exchange activity or oxidation of -SH groups in rat liver microsomes. The enzyme activity was assayed with various concentrations of CdCl_2 in the absence (\circ) or presence (\bullet) of 1 mM DTT. The number of -SH groups was measured by Ellman reaction (\blacktriangle). Representative results obtained for three different membrane preparations are shown; they varied by 4–7%.

bate was fully reversed in the presence of BHT (Fig. 2A), in parallel with complete prevention of TBARS formation (Fig. 2B). The time-dependent inactivation of both the ethanolamine BE enzyme and G-6-Pase by Fe^{2+} /ascorbate was delayed by 2 min in the presence of 30 mM thiourea, another specific hydroxyl radical scavenger [10], with no further effect on the velocity of TBARS formation (not shown). These results suggest that modification of sulfhydryl group(s) is not directly involved in inactivation of the ethanolamine base exchange enzyme, as has been shown for G-6-Pase [30,31].

Since a striking positive correlation has been found between TBARS formation, -SH group oxidation, and inhibition of the BE enzyme activity (Fig. 2D), it can be concluded that the primary effect of Fe^{2+} /ascorbate on the ethanolamine BE reaction is propagated via peroxidation of lipids, and that the effect of changes in redox state of microsomal membranes is a rather secondary factor. When the preincubation medium was supplemented with 1 mM DTT, after 10 min of preincubation at 37°C , Fe^{2+} /ascorbate inhibited the ethanolamine BE activity by 46%, while in the absence of DTT it inhibited it by 92% (Fig. 2A). At the same time, 0.07 nmol of TBARS per mg of

protein was formed in the presence of 1 mM DTT, whereas 0.85 nmol was formed in its absence (Fig. 2B). Note that 1 mM DTT slightly inhibits the enzyme activity (Fig. 2A, see also the next paragraph).

It has to be stressed that MDA directly added (up to 3.2 nmol per mg protein) to the ethanolamine BE assay medium only slightly inhibited PE formation at a rate of $0.008 \text{ pmol PE} \times \text{min}^{-1} \times \text{mg}^{-1}$ per pmol MDA added per mg protein. The rate of inhibition of the reaction in the presence of endogenously formed MDA (TBARS) was over 20 times higher, calculated from the linear part of the plot of BE activity inhibition versus TBARS formation (see Fig. 2D). This raises the question of possible effectors involved in the mechanism of inhibition of the ethanolamine BE activity by prooxidants, especially the importance of the level of lipid hydroperoxides and aldehydes (for example 4-hydroxynon-2-enal). It is well known that 4-hydroxyalkenals block thiol groups of peptides, thus preventing formation of disulfide bridges essential for the enzymatic activity of integral ER proteins [11–13,30].

A significant reduction (by 30%) of both inhibition of the ethanolamine BE activity and stimulation of TBARS formation by Fe^{2+} /ascorbate was observed in the presence of 1 mM CaCl_2 in the preincubation medium. It can be suggested that calcium ions, by interacting probably with anionic oxygen in phosphate group of PS molecules, slow down formation of lipid peroxide radicals within the membrane. Therefore, PS molecules may play a pivotal role as starting points of the phospholipid peroxidation cascade. Indeed, Tampo and Yonaha [16] have found promotion of liposomal 1-stearoyl-2-arachidonoyl-PC peroxidation in the presence of Fe^{2+} -chelate when liposomes were enriched with dipalmitoyl-PS. On the other hand, 1-palmitoyl-2-linoleoyl-PE added to liposomes reduced lipid peroxidation. Taken together, the cited and our own observations led us to the conclusion that the ethanolamine BE reaction may be responsible for synthesis of the important molecular species of PE involved in the cell self-defense against lipid peroxidation. This does not exclude the possibility that ferrous cations interacting with nitrogen ligands [32] in $-\text{NH}_2$ groups may directly affect the enzyme activity.

3.3. Effect of the -SH group modifying reagents on ethanolamine BE activity

To establish the effect of redox potential on PE synthesis via the BE reaction, several -SH group modifying reagents were examined. As shown in Table 1, the effect of these reagents was dependent on their chemical structure and hydrophobicity. Mersalyl, a non-penetrating reagent, had no influence on BE activity, while water-soluble DTT, penetrating to

Table 2
NADPH-dependent lipid peroxidation and synthesis of PE via the base exchange reaction

| Effector | Concentration (μM) | Enzyme activity (% of control) | TBARS formation ($\text{pmol} \times \text{mg}^{-1} \text{ protein}$) |
|-------------------------------------|---------------------------------|--------------------------------|---|
| FeCl_3 | 50 | 121 | 137.2 |
| $\text{Fe}_3(\text{SO}_4)_2$ | 50 | 100 | 99.4 |
| FeSO_4 | 20 | 45 | 225.3 |
| NADPH | 250 | 78 | 130.9 |
| NADPH+ FeCl_3 | 250/50 | 40 | 149.7 |
| NADPH+ $\text{Fe}_3(\text{SO}_4)_2$ | 250/50 | 15 | 147.5 |
| NADPH+ FeSO_4 | 250/20 | 6 | 338.6 |

Microsomes were preincubated with the effector(s) listed for 10 min at 37°C . Enzyme activity and TBARS level in control membranes amounted to

some extent into the lipid bilayer, inhibited it (IC_{50} 3.4 mM), even at concentrations (1–2 mM) routinely used by other investigators in the assay medium. Due to its low redox potential (-0.33 V at pH 7.0), DTT is able to maintain thiol groups of proteins completely in the reduced form [33]. On the other hand, lipophilic pCMB, intercalating the lipid bilayer [34] and inhibitory to NADPH-cytochrome P450 reductase [16], completely abolished the ethanolamine BE activity with IC_{50} of 130 μ M; so also did NEM, known to freely penetrate the membrane bilayer (IC_{50} 1.6 mM). Neither of the thiol reagents used affected the amount of TBARS formed (Table 1).

Unlike Fe^{2+} , cadmium ions, known inhibitors of dithiols, interact mainly with sulfur ligands [32], and do not stimulate TBARS formation (Table 1). The observed strong inhibition of ethanolamine BE activity by Cd^{2+} with an IC_{50} of 13 μ M (Fig. 3) was not prevented by the addition of BHT, but was fully reversible in the presence of 1 mM DTT, which easily chelates metal ions [33] and protects -SH groups from oxidation. It is worth mentioning that Cd^{2+} used in concentrations close to the IC_{50} for the ethanolamine BE activity inhibition had but a slight effect on the overall number of -SH groups in microsomal membranes (Fig. 3). Therefore, the effect of cadmium ions can be explained in terms of their interaction with the BE enzyme calcium binding sites or of the effect of Cd^{2+} on the essential -S-S- bridges within a protein molecule.

3.4. NADPH-dependent lipid peroxidation and PE synthesis via the BE reaction

In a reconstituted system composed of microsomal cytochrome P450 reductase/cytochrome P450 and Fe^{3+} , NADPH initiates lipid peroxidation via the well-established pathway of creation of highly reactive superoxide radical anion ($O_2^{\cdot-}$) [16,17]. Addition of NADPH to rat liver microsomal membranes caused a 20% inhibition of the ethanolamine BE activity (Table 2), without affecting the linear dependence of the enzymatic reaction on the reaction time. Concomitantly, formation of TBARS has been observed. Ferric cations, which have no influence on the enzyme activity (Table 2, see also Section 3.2), upon reduction to Fe^{2+} in the presence of NADPH became highly inhibitory to the phospholipid synthesis, and their effect was additive with that of NADPH. This suggests that reduction of endogenous Fe^{3+} present in the catalytic center of the microsomal metalloproteins (cytochrome P450 reductase/cytochrome P450) inhibited the ethanolamine BE activity due to stimulation of lipid peroxidation, but also due to the direct oxidative damage of enzymatic protein (mainly production of carbonyl groups) [17]. Moreover, exogenous Fe^{2+} and NADPH synergistically inactivated the ethanolamine BE enzyme, and initiated TBARS formation (Table 2). The lower extent of TBARS formation in the presence of Fe^{2+} /NADPH than of Fe^{2+} /ascorbate (Fig. 2B) can be explained by the fact that a microsomal iron-ascorbyl radical complex acts as a catalyst in the initiation of lipid peroxidation, without ascorbate being oxidized during the process [35]. On the other hand, NADPH at pH 7.4 is efficiently oxidized, therefore, its effect on the TBARS formation is lower.

The results of the present investigation seem to favor the hypothesis that there is a close relationship between cytochrome P450 or cytochrome P450-dependent proteins and the phospholipid base exchange reaction. The latter reaction is highly sensitive to the redox state of membrane, which, in

turn, is regulated by the above-mentioned enzymes. The induction of specific isoforms of cytochrome P450 (namely CYP4A) by the hypolipidemic agent, clofibrate [36], is accompanied by an increase in the ethanolamine BE activity in ER membranes isolated from drug-treated rats (Lenart, J., Komańska, I., Pikuła, S. and Jasińska, R. (1996) submitted). On the other hand, PE and PS synthesized via the BE reaction are indispensable for cytochrome P450 activity [7]. In addition, it is well established that in vivo the synthesis of phospholipids via the BE reaction is sensitive to several compounds, for example antimycotics, known to be cytochrome P450 inhibitors [37].

The presented results concerning the sensitivity of the ethanolamine BE reaction to prooxidants and factors changing the redox state of microsomal membranes may have more general implications with respect to cellular processes related to metabolic and oxidative stress. Endoplasmic reticulum of hepatocytes, where that reaction is localized, is the major site of lipid synthesis for all other organs, as well as for other cellular compartments. The molecular species of phospholipids containing unsaturated or polyunsaturated fatty acid moieties in their *sn*-2 position are the main targets of highly reactive oxygen radicals and, in consequence, constitute a rich source of lipid hydroxyperoxides. The latter compounds, by a self-propagating process, may cause further oxidative damage to many cellular systems and biologically important molecules [38]. The characteristic feature of the phospholipid BE reaction studied in this report is that the above-mentioned molecular species of phospholipids serve both as membranous substrates and reaction products. Their unique physicochemical properties point to the importance of these molecules not only for a proper environment for membrane proteins but, first of all, for regulating many vital cellular processes like enzymatic catalysis, transport of solutes or signal transduction. PE and PS also belong to these phospholipid classes which are most sensitive to oxidative damage. In analogy to the proposed antioxidative mechanism of α -tocopherol action [39], it can be suggested that particular molecular species of both phospholipids are localized close to the enzymes that participate in the microsomal electron transport system. PE and PS are the source of polyunsaturated fatty acids for the cell and are synthesized mainly by the BE reaction. Therefore, one may speculate that, when their level is decreased upon oxidative stress, the reaction of phospholipid base exchange is switched on to restore their physiological concentrations in a membrane. If so, this reaction may represent a superfine tuning up system regulating the content of specific molecular species of PE and PS in biological membranes.

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