Phosphatidylcholine synthesis-related enzyme activities of bovine brain microvessels exhibit susceptibility to peroxidation

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Abstract In microvessels isolated from bovine brain, microsomal enzyme activities involved in phosphatidylcholine biosynthesis and degradation were determined. The microvessels possessed acyl-CoA:1-acyl-*sn*-glycero-3-phosphocholine (AT) and glycerophosphocholine phosphodiesterase (GroPChoPDE) activity at a higher level compared with bovine and rat brain or rat liver microsomes whereas they expressed CTP:phosphocholine cytidylyltransferase (CT) and choline phosphotransferase (CPT) activity at a lower level. Each enzyme has been characterized in terms of response to inhibitors or activators revealing properties very similar to those in brain and liver microsomes. In the homogenate prepared from *t*-butylhydroperoxide-treated microvessels (10 min exposure to 10 μM up to 1 mM concentrations), AT and CPT activities exhibited a significant dose-dependent inhibition. In contrast, GroPChoPDE activity was unaffected. CT was inhibited only at 1 mM concentration. Short treatment of microvessels with Fe^{2+} (20 μM)-ascorbate (0.25 mM) or 100 μM linoleate hydroperoxide did not have any effect on the activity of the four enzymes. Strong inhibition of all enzymes was noted when the linoleate hydroperoxide system was fortified by Fe^{2+} ions (100 μM). AT inactivation was also found when oxidized low density lipoprotein was preincubated with microvessels. On the other hand, oxidized LDL left unchanged CPT and GroPChoPDE activities whereas it promoted a slight stimulation of cytidylyltransferase activity. Overall, the results suggest a link between oxygen radical generation and the perturbation of the microvessel membrane structure in which the four enzymes are incorporated, coupled to a direct sulfhydryl protein modification.

Key words: Lipid peroxidation; Phosphatidylcholine; Brain microvessel; Endothelial cell; Phospholipid enzyme

1. Introduction

The blood-brain barrier, which is composed of capillary endothelial cells, is involved in the aging and disease process. There are many indications that its disruption is one of the early steps in the pathogenesis of multiple sclerosis, Alzheimer's disease, brain tumors, hypertension, bacteraemia, seizures, hepatic coma, ischemia and vasogenic brain edema [1–3]. It is thought that disruption may occur because of a combination of increased intercellular leakage caused by the opening of tight junctions and enhanced pinocytosis in brain microvessels.

In order to understand the biochemical basis of normal transendothelial transport and its dysfunction during diseases or inflammation, we started to investigate the presence and characteristics of enzymes involved in phosphatidylcholine (PtdCho) metabolism in crude microvessel preparations from bovine brain. The reasons for undertaking these biochemical studies are two-fold: (a) isolated microvessels offer the advantage of dealing with easy preparations of an intact three-dimensional structure and not dedifferentiated cell populations. On the contrary, they have the disadvantages of abluminal pericyte, arteriolar smooth muscle cell and astroglial endfeet contamination; (b) in all types of microvessels or in cultured endothelial cells, the occurrence and key role of CTP:phosphocholine cytidylyltransferase (CT), choline phosphotransferase (CPT) and glycerophosphocholine phosphodiesterase (GroPChoPDE) for the synthesis and remodeling of their major membrane phospholipid [4] were until now believed to be putative.

Consistent with the notion that oxidative stress plays an important role in atherogenesis, in the present paper we explored the linkage between diverse oxidant injuries and the induction of acute changes in the regulatory enzymatic mechanisms of phospholipid metabolism in the brain microvasculature. The protective effect, if any, of antioxidants on peroxidative inactivation of membrane-bound acyltransferase was also tested. Our observations may support the hypothesis that the effect of penetrable oxidative signals directly mediate deep inhibition or slight activation of some membrane-bound phospholipid enzymes.

2. Materials and methods

2.1. Isolation of bovine brain microvessels

The isolation of bovine cerebral microvessels was performed using a slight modification [5] of the method of Hjelle et al. [6]. The enrichment of microvessel preparation, determined by measurement of alkaline phosphatase [7] and γ-glutamyltranspeptidase [8], was between 20- and 28-fold over the bovine brain homogenate. At the end of the purification process, dispersed microcapillaries were examined by phase contrast microscopy.

2.2. Preparation of pro-oxidant systems

Purified hydroperoxylinoleate was prepared as previously described [9,10]. Low density lipoprotein from human plasma was prepared following the procedure of Chung et al. [11], and autoxidized by dialysis against EDTA-free isotonic saline supplemented with 10 μM $CuSO_4$ at 4°C for 24 h [12]. The degree of oxidation of the LDL preparations was determined by measuring the hydroperoxide content according to Gebicki and Guille [13].

2.3. In vitro peroxidation of microvessels

Microvessel preparations were extensively washed with 122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 1.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.4 mM K_2HPO_4, 10 mM glucose and 10 mM Hepes buffer, pH 7.4, suspended in 0.9% NaCl corrected to pH 7.4, and diluted to a protein concentration of 14 mg/ml. In preincubation steps, freshly

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prepared microvessels were treated under air at 37°C for 10 min with pro-oxidants as follows:

(a) Serial amounts of *t*-butylhydroperoxide (*t*-BuOOH), employed to generate *t*-butoxyl or *t*-butylperoxyl radicals mainly by the action of intracellular glutathione selenoperoxidases, were added (0.01–1 mM, final concentration) to microvessel preparations. The resulting suspensions were vortexed and incubated.

(b) A saline (0.9% NaCl) solution of Fe^{2+} (20 μM, final concentration)-ascorbate (0.25 mM, final concentration), employed to generate hydroxyl radicals, was added to aliquots of microvessel preparations, and the resulting suspensions were vortexed and incubated [9].

(c) Microvessels were incubated with either purified linoleate hydroperoxide (0.25–2 mM) or linoleate hydroperoxide (2 mM) potentiated by Fe^{2+} (100 μM) (see [10]).

(d) Microvessels were incubated with control or oxidized LDL (1 mg protein/0.5 ml incubation medium).

Control microvessel incubations were run simultaneously, and always performed without the addition of peroxidation inducers. Lipid peroxidation was assessed by malondialdehyde (MDA), conjugated diene (CD) and lipid hydroperoxide level measurements (see [10,14]). Protein carbonyl and thiol group content were also used as indices of oxidative damage of membrane proteins [15]. At the end of all the incubation procedures, control and peroxidized microvessels were washed twice with saline, and immediately used for enzyme activity determinations. None of the pro-oxidant-treated preparations showed cell lysis phenomena by observing a limited number of samples under phase contrast microscope. No release of enzyme activities was detected in washings, even in the presence of cytidylyltransferase lipid activators (PtdCho 2 mg/ml, and 0.1 mM oleate).

2.4. Enzyme assays

Enzyme activities were determined after homogenization of washed control and pro-oxidant-treated microvessels with 20 mM Tris-HCl, pH 7.4, in a Potter-Elvehjem motor-driven apparatus. The homogenate was used for measurements of enzyme activities. Membrane protein determinations were performed according to Lowry et al. [16].

All enzyme determinations were performed under optimal conditions. Acyl-CoA:1-acyl-*sn*-glycero-3-phosphocholine *O*-acyltransferase (EC 2.3. 1.23) was assayed using 25 μM [1-^{14}C]oleoyl-CoA (Amersham Int., UK) and 50 μM lysophosphatidylcholine as substrates, as previoulsy described [17], except that the incubation buffer was 70 mM Tris-HCl buffer, 1 mM EDTA, 5 mM DTT, pH 7.4. After 10 min incubation, the reaction product (phosphatidylcholine) was estimated radiometrically following thin-layer chromatography separation of the lipid extract. The inhibitors to which the enzyme was exposed were *N*-ethylmaleimide and *p*-chloromercuribenzoate, under conditions described in the legend to Fig. 3. CTP:phosphocholine cytidylyltransferase (EC 2.7.7.15) was assayed radiometrically according to Jamil and Vance [18], using phospho[methyl-^{14}C]choline (Amersham Int., UK) as substrate (1.5 mM). The inhibitors to which the enzyme was exposed were *N*-ethylmaleimide and zinc ions, under conditions described in the legend to Fig. 3. Microsomal choline phosphotransferase (EC 2.7.7.2) was measured according to Van Golde et al. [19] with slight modifications, using CDP[methyl-^{14}C]choline as radioactive substrate and 1,2-diacylglycerols prepared by treatment of bovine brain PtdCho with phospholipase C [20]. The effects to which the enzyme was exposed were those of phospholipase C and phospholipase A_2, under conditions described in the legend to Fig. 3. Microsomal GroPChoPDE (EC 3.1.4.2) was measured as described previously [21]. The inhibitors to which the enzyme was exposed were EDTA and L-α-glycero-3-phosphate, under conditions described in the legend to Fig. 3.

To assess the effect of antioxidants on enzyme inactivation, αtocopherol, butylated hydroxytoluene (BHT) and catalase were added to microvessel homogenates incubated with 1 mM *t*-BuOOH. At the end of incubation period (10 min at 37°C), the homogenates were used for the estimation of acyltransferase activity which was taken as paradigm of an enzyme susceptible of inactivation.

In a set of preliminary experiments, ethanolamine phosphotransferase (EC 2.7.7.1) was assayed according to the same procedures as the choline derivative, using CDP[1,2-^{14}C]ethanolamine as substrate (ICN Biomedicals, Inc., CA). GroPEtnPDE assays was carried out by the spectrophotometric method described by Baldwin and Cornatzer [22].

3. Results

Fig. 1 shows the in vitro effect of various free radical generating systems on bovine brain microvessels. MDA formation markedly increased after incubation with peroxyl (*t*-BuOOH or hydroperoxides) or hydroxyl (Fe^{2+}/ascorbate) radical inducers, even during incubation with pure linoleate owing to the presumable presence of metal ion impurities. CD levels in microvessels, 10 min after incubation with 10 μM or 1 mM *t*-BuOOH, were augmented 2–3-fold compared to the control, whereas they increased 8-fold with linoleate hydroperoxide/Fe^{2+} system. Lipid hydroperoxide levels increased 2-fold in oxidized LDL-treated microvessel preparations, and

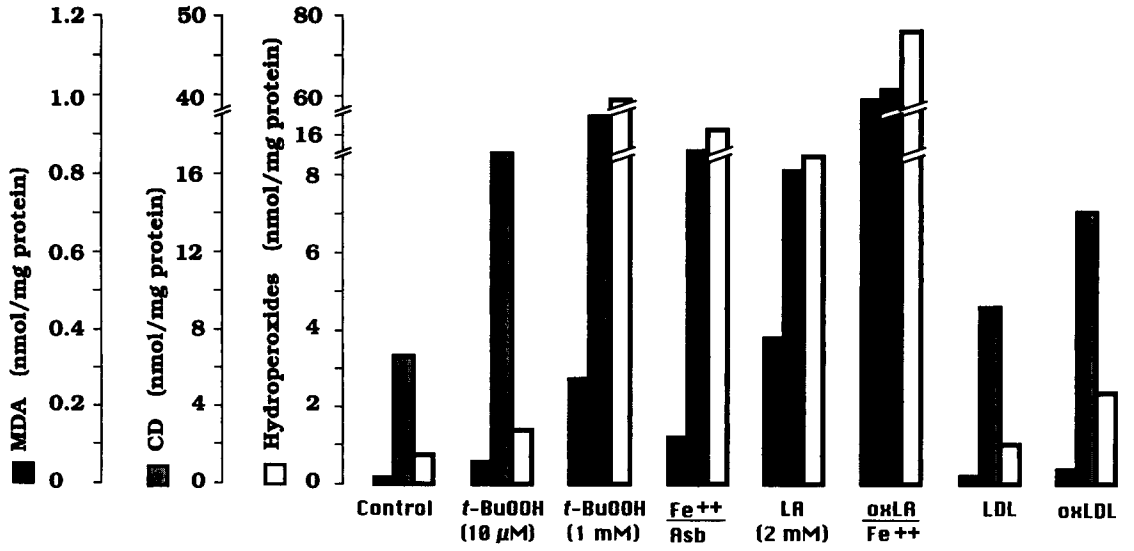

Fig. 1. Malondialdehyde (MDA), conjugated diene (CD) and hydroperoxide levels of microvessels freshly isolated from bovine brain and incubated in air, at 37°C for 10 min, in a medium containing 0.9% NaCl, corrected to pH 7.4, and soluble peroxidation inducers added at the final concentrations indicated in section 2. Afterwards the intact microvessels were aliquoted and separately processed for the determination of peroxidation indices. Each data bar is the mean of two independent experiments in which the variation was less than 20%. Asb, ascorbate; LA, linoleate; oxLA, oxidized linoleate.

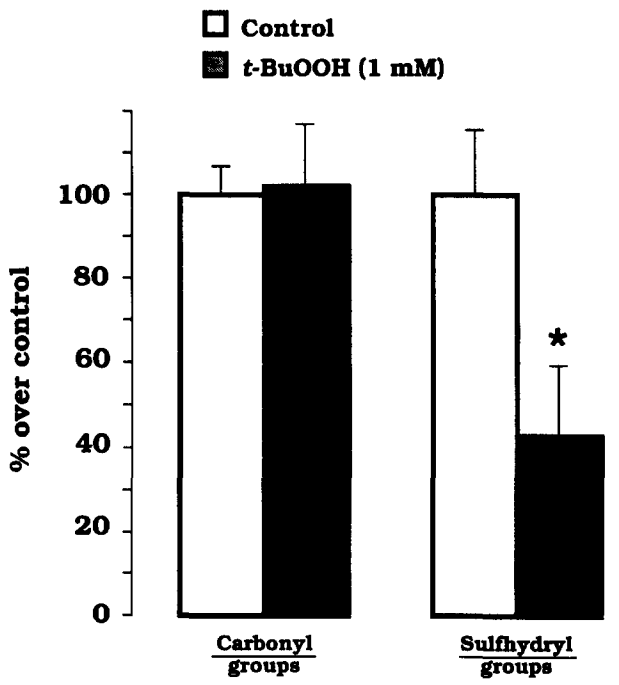

Fig. 2. Effect of 1 mM *t*-BuOOH on the carbonyl- and sulfhydryl-group content of proteins in bovine brain microvessels. Homogenates of microcapillaries were incubated with the prooxidant for 10 min at 37°C as described in section 2. Protein carbonyl groups were measured in the protein pellet obtained after homogeneization with ice-cold 20% trichloroacetic acid, centrifugation at 14000 rpm for 15 min and suspension in 2 N HCl. Protein sulfhydryl groups were measured in the protein pellet obtained after homogenization in ice-cold 0.3 M $HClO_4$ containing 5 mM EDTA, centrifugation at 14000 rpm for 15 min and solubilization in 1 ml of 6 M guanidine hydrocloride dissolved in 0.05 M KH_2PO_4, 5 mM EDTA, pH 7.4. Each data bar represents the mean of three individual microvessel samples. *Significantly different from incubated unoxidized controls.

more drastically in the other systems, for instance 90-fold using oxidized linoleate-Fe^{2+}.

Fig. 2 shows the in vitro effect of *t*-BuOOH on protein oxidation of bovine brain microvessels. This pro-oxidant, taken as a representative example of pro-oxidant system which generates rapid and intensive lipid peroxidation, at 1 mM concentration was able to induce a decrease in protein sulfhydryl groups. No significant formation of protein carbonyl groups was observed.

The effect of specific inhibitors or activators on AT, CT, CPT and GroPChoPDE activities is reported in Fig. 3. We found that the inhibitory effects or the stimulatory effect on CPT activity induced by phospholipase C treatment (accumulation of endogenous diacylglycerols) were similar to those previously measured in rat brain or liver microsomes [21,23].

Table 1 shows that the activity of both AT and CT markedly decreased after short microvessel incubation with high concentrations of the membrane-permeant oxidant *t*-BuOOH. The activity changes induced by either Fe^{2+}/ascorbate or 100 μM linoleate hydroperoxide were not significant. Acyltransferase specific activity was strongly inhibited (15-fold maximum loss) by 2 mM linoleate whereas cytidylyltransferase remained unaffected. The presence of Fe^{2+} ions in the incubation system together with 2 mM linoleate hydroperoxide abolished the acyltransferase activity and caused a 5-fold decrease in cytidylyltransferase specific activity. The dramatic effect may be due to the association of the detergent effect of linoleate hydroperoxide and the generation of bilayer-penetrating, self-perturbating lipoperoxyl radicals overwhelming the glutathione peroxidase reducing system. Microvessel AT was half-reduced whereas CT was significantly stimulated (84% activity increase over control) by oxidized LDL treatment.

Table 1 shows that all in vitro peroxidation models brought about changes of microvessel choline phosphotransferase specific activity quite similar to those observed for acyltransferase. Moreover, as in the CT experiments, GroPChoPDE specific activity was inhibited 10-fold in microvessels which were incubated with linoleate hydroperoxide plus Fe^{2+} mixture. All other peroxidation inducers were ineffective.

The enzymes related to phosphatidylethanolamine metabolism here tested, namely ethanolamine phosphotransferase and glycerophosphorylethanolamine phosphodiesterase, showed extremely low or undetectable baseline activity (data not shown).

The effects of various antioxidants and free-radical scavengers on lipid peroxidation and AT activity were compared in *t*-BuOOH system. Table 2 lists the compounds used and their final concentrations. Lipid scavenger BHT and α-tocopherol are able directly to detoxicate *t*-BuOOH thereby inhibiting lipid peroxidation (63 and 27% decrease in MDA production). Catalase failed to protect microvessels against *t*-BuOOH effects. This result was expected since *t*-BuOOH is not metabolized by catalase [24].

4. Discussion

The presence of acyl CoA:lysophosphatidylcholine acyltransferase in the endoplasmic reticulum of cultured arterial endothelial cells has been demonstrated [25,26]. To our knowledge, no information concerning the other three mem-

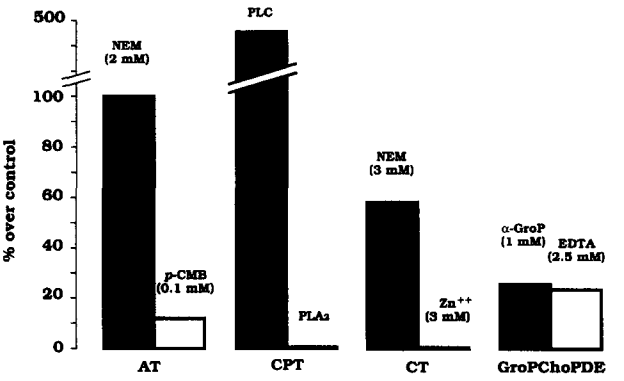

Fig. 3. Effect of specific inhibitors and activators on AT, CPT, CT and GroPChoPDE activities associated with bovine brain microvessel preparations. Values are mean percentages over control of residual activity from two separate experiments. Conditions of standard assays were described in section 2. *N*-Ethylmaleimide (NEM), *p*-chloromercuribenzoate (p-CMB) and EDTA were added to microvessel preparations which were then preincubated for 10 min at 37°C. For CT assays, the microvessel preparations were preincubated with NEM or Zn^{2+} for 10 min at 4°C, in the absence of CTP. L-α-Glycerol-3-phosphate (α-GroP) was added to enzyme assay mixtures without preincubation. Microvessel homogenates were incubated with phospholipase C (PLC) from *Clostridium perfrigens* (8 units) or phospholipase A_2 (PLA_2) from bee venom (8 units) in 50 mM Tris-HCl buffer containing 2.5 mM $CaCl_2$ (pH 7.5) at 37°C for 30 min with intermittent shaking, and processed as previously described [18].

Table 1
Effect of pro-oxidants on the acyl CoA:1-acyl-*sn*-glycero-3-phosphocholine *O*-acyltransferase, CTP:phosphorylcholine cytidylyltransferase, choline phosphotransferase (CPT) and glycerophosphocholine phosphodiesterase (GroPChoPDE) activity of isolated bovine brain microvessels

Additions	Activity (nmol/min per mg protein)			
	Acyltransferase	Cytidylyltransferase	CPT	GroPChoPDE
None	1.29 ± 0.23	0.21 ± 0.04	0.134 ± 0.03	4.87 ± 1.2
t-BuOOH (10 μM)	0.98 ± 0.29	0.25 ± 0.04	0.121 ± 0.03	5.07 ± 1.6
t-BuOOH (100 μM)	0.14 ± 0.03[e]	0.20 ± 0.03	0.075 ± 0.02[d]	3.40 ± 1.6
t-BuOOH (1 mM)	0.07 ± 0.01[e]	0.02 ± 0.004[e]	0.002 ± 0.00[e]	3.57 ± 0.7
Fe^{2+}(20 μM)/Asb (250 μM)[a]	1.27 ± 0.32	0.19 ± 0.03	0.115 ± 0.03	4.40 ± 0.9
Linoleate (100 μM)	1.32 ± 0.30	0.21 ± 0.03	0.140 ± 0.03	4.73 ± 1.0
Linoleate (2 mM)	0.09 ± 0.02[e]	0.20 ± 0.02	0.045 ± 0.01[e]	4.82 ± 1.5
Linoleate hydroperoxide (100 μM)	1.48 ± 0.29	0.19 ± 0.02	0.120 ± 0.03	4.19 ± 0.6
Linoleate hydroperoxide (2 mM)/Fe^{2+} (100 μM)	0.02 ± 0.01[e]	0.04 ± 0.01[e]	0.010 ± 0.003[e]	0.46 ± 0.1[e]
Native LDL[b]	1.15 ± 0.27	0.33 ± 0.05	0.120 ± 0.02	3.77 ± 0.9
Peroxidized LDL[c]	0.52 ± 0.09[e]	0.38 ± 0.03[d]	0.117 ± 0.02	3.99 ± 0.8

Incubations of microvessels (7 mg protein/0.5 ml) were conducted in a medium containing 0.9% NaCl, corrected to pH 7.4, following 10 min exposure at 37°C to the indicated conditions.
Values represent the mean ± S.D. of 3–6 separate preparations. By ANOVA, [d]$p<0.05$ or [e]$p<0.001$ for significant difference from untreated controls.
[a]Microvessels, preincubated in 20 μM Fe^{2+}, 250 μM ascorbic acid (Asb), with the addition of 20 μM 8-hydroxyquinoline at 37°C for 10 min, were also exposed to 50 μM H_2O_2 for an extra period of 10 min [35,36]. No significant changes of peroxidation indices and enzyme activity mean values were detected (data not shown).
[b]1 mg protein/0.5 ml incubation medium.
[c]Peroxidized low density lipoprotein contained 75 nmol of hydroperoxides/mg protein.

brane-bound enzyme activities has previously been available in the literature. In the crude homogenate of isolated bovine brain microcapillaries, we demonstrated the occurrence of AT and GroPChoPDE at a higher level of activity compared with bovine and rat brain or rat liver microsomes which express CT and CPT at a lower level of activity (see [14,19,21]). AT activity seems to meet the needs of a rapid reacylation of intracellular lysoPtdCho which, as shown by a recent finding of ours [27], is avidly transported across the luminal side of blood-brain barrier at a rate even higher than that of free fatty acids. The very high GroPChoPDE activity was surprising. This finding does not occur with other tissue preparations except kidney microsomes in which the enzyme is particularly enriched [22]. The significance of this considerable degradative activity upon glycerophosphocholine, a catabolic product of PtdCho [28], remains obscure at this point.

External pro-oxidants generate cellular damage as a result of their interaction with lipids, proteins, nucleic acids and carbohydrates. Oxidative modifications of proteins can involve direct fragmentation, loss of thiol groups or may provide denatured substrates for intracellular proteolysis [29,30]. The susceptibility of microvessel proteins to reactive oxygen species generated by *t*-BuOOH, as a representative example of pro-oxidant system which generates rapid and intensive lipid peroxidation, has been tested in this study. The results in Figs. 1 and 2 indicate a general sensitivity of microvessel lipids and proteins to oxygen radicals.

The results obtained with the peroxidation experiments are

Table 2
Effect of antioxidants on lipid peroxidation and acyltransferase activity in isolated bovine brain microvessels

Additions	Activity (nmol/min per mg protein)	
	MDA	Acyltransferase
None	N.D.	1.46
BHT (100 μM)	N.D.	2.18
catalase (5 mg/ml)	0.10	1.33
t-BuOOH	0.40	0.76
t-BuOOH+BHT (100 μM)	0.15	1.51
t-BuOOH+catalase (5 mg/ml)	0.34	1.44
None sonicated	N.D.	1.63
α-Tocopherol (300 μM)	N.D.	1.55
t-BuOOH	0.37	0.97
t-BuOOH+α-tocopherol (300 μM)	0.27	1.53

Microvessel homogenates (2 mg protein/ml) with 20 mM Tris-HCl, pH 7.4, were pretreated with catalase or butylated hydroxytoluene (BHT, 10 μl ethanolic solution) at 37°C for 2 min, and exposed to 1 mM *t*-BuOOH for an additional 8 min. Ethanolic stock solutions of α-tocopherol were added to glass test tubes and evaporated under N_2. To the resulting, thin, dry film was added 20 mM Tris-HCl buffer pH 7.4, and the lipid-soluble antioxidant dispersed by sonication with a Vibracell sonicator at 30% power output for 2 min at 4°C. Microvessel homogenates with 20 mM Tris-HCl, pH 7.4, were then added to achieve a final concentration of 2 mg protein/ml. The mixtures were sonicated again for 4 min at 4°C to ensure proper dispersion and incorporation of α-tocopherol into membranes, and exposed to 1 mM *t*-BuOOH for an additional 10 min at 37°C. Aliquots of incubation mixtures were used either for MDA assays or AT activity determinations as described under section 2. In control (10 min preincubation with no additions), peroxidized and antioxidant-treated microvessel homogenates, AT assays were performed after washing the homogenates twice with 20 mM Tris-HCl buffer. Values represent the mean obtained from two separate preparations. N.D., not detectable.

consistent with those reported by our laboratory, showing inhibition of phospholipid-dependent AT and a lack of alteration of amphitropic CT function in axolemma-enriched fractions or brain microsomes [9,14]. In addition, phospholipid-dependent CPT seems to behave like AT, and GroPChoPDE like CT. The data again support the notion that the nature of hydrophobic moieties (hydroperoxy, hydroxy or unoxidized acyl chains) of phospholipids in the vicinity of the enzymatic membrane protein is not an important structural determinant for expressing CT activity. When the fluidity of the membrane phospholipids decrease, due to peroxidation events, the CT molecule might keep sulfhydryl residues protected and become more accessible to hydrophilic substrate. Pro-oxidants may induce loss of buried thiol groups on the protein and/or destroy membrane phospholipid organization only at very high concentrations (1 mM *t*-BuOOH or 2 mM linoleate hydroperoxide).

The slight stimulatory effect on CT activity observed with oxidized LDL may be due to the lipid peroxides carried by oxidized lipoprotein which are able to generate potential activating lysophospholipids [31] during membrane component peroxidation. Alternatively, the translocation of CT activity from cytosol following LDL receptor binding, independently of the presence of oxygen-based radicals on LDL, could be hypothesized. Investigation of this effect by more appropriate in vitro experiments (cultured endothelial cells) which overcome the poor permeability of external LDL through abluminal surface of intact microvessels is needed. In fact, by using crude microvessel preparations our preliminary data indicated a lack of dose dependency of LDL stimulatory effect.

The slight activatory effect of native LDL on CT may be related to the increase in the release of O_2^- and NO production by endothelial cells directly challenged by native LDL [32].

The insensitiveness of GroPChoPDE to oxidant injuries, except the more dramatic ones (high concentration of hydroperoxylinoleate plus Fe^{2+}), indicates that enzyme active site does not possess easily oxidable groups (key sulfhydryl). It may also be well protected from compositional and conformational changes of lipid moieties of the surrounding, less fluid bilayer following their oxidative shortening and/or hydroxy/peroxy chemical modifications. The nature of membrane embedding and the structure of GroPChoPDE are at present unknown.

Whereas lipid and organic hydroperoxides appear to be responsible for alteration of the enzyme activities studied here, we surprisingly found no evidence that Fe^{2+}/ascorbate system (a very effective oxidant under acellular conditions [9,14,33]) has any effect in inducing enzymatic changes. This may be interpreted as indicating that generated hydroxyl radical cannot penetrate basal and cell membrane of intact microvessels. Alternatively, endothelial cells and pericytes possess enough antioxidants and scavenger enzymes [34] to oppose the mild, in progress oxidative stress induced by Fe^{2+} ions.

Lipid-soluble α-tocopherol and non-thiol antioxidant BHT did not fail to prevent lipid peroxidation, directly reacting with *t*-BuOOH. Since they and/or their oxidation products (stable phenolic radical or delipidating *t*-butyl alcohol, still lipidic in nature) also possess properties of inhibiting membrane-bound AT, it is essential to perform a washing procedure upon membranes before determining AT. This enzyme molecule is very susceptible to the disorganization of membrane structure caused by intercalating lipophilic compounds, detergents, local anesthetics, aliphatic alcohols [37–39].

From our results we conclude that free radicals induce profound changes in key enzyme activities related to PtdCho turnover in brain microvessel membranes. This may contribute to the impairment of capillary endothelial cell and pericyte mechanisms that maintain electrolyte and water homeostasis or sustain substrate transport to the brain. The present study is introductory to the one aimed at investigating whether astrocytes are able to induce over-expression of phospholipid enzymes or re-expression of kinetic characteristics of such enzymes eventually down-regulated after a few passages in culture. To this end, we are presently carrying out an in vitro study of the modulation of PtdCho metabolism as the cofactor in establishing tight junctions among endothelial cells of the brain microvessels.

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