BIOPHYSICS AND BIOCHEMISTRY

Palmitoylcarnitine, an Important Component of the Repair System in the Synaptosome Membrane, in Oxidative Stress

Yu. Yu. Tyurina, A. Arduini,* V. A. Tyurin, T. V. Sokolova, and E. Arrigoni-Martelli**

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Oxidative stress is accompanied by a considerable rise of palmitic acid incorporation into phosphatidylethanolamine of synaptosome membranes due to activation of lysophosphatidylethanolamine palmitoyltransferase and carnitine palmitoyltransferase. In the absence of ATP carnitine palmitoyltransferase plays an essential role in reacylation of phosphatidylethanolamine, and palmitoylcarnitine serves as a reserve pool of palmitoyl coenzyme A.

Key Words: *synaptosomes; oxidative stress; palmitoylcarnitine; reacylation*

Oxidative stress observed in various pathologies of the central nervous system (brain ischemia and hypoxia, Alzheimer disease, etc.) is accompanied by activation of free radical processes and deacylation reactions and results in accumulation of products of lipid peroxidation (LPO) and phospholipid hydrolysis (free fatty acids and lysophospholipids) [7,9-13].

It should be noted that polyene fatty acids in phospholipid molecules are the main substrate of deacylation reactions catalyzed by phospholipase A_2 [7,13]. Under these conditions, deacylation, apart from its destructive action, can exert a protective effect due to elimination of oxidized fatty acid from phospholipid molecule [13]. However, lysophospholipids remaining in the membrane represent a real danger of modification and damage to membranes due to their detergent action [10]. Lysophospholipids are utilized in the reaction of reacylation catalyzed by ATP-dependent acyl coenzyme A (CoA)-synthetase and lysophospholipid acyltransferase [10,13]. Activation of free radical processes is accompanied by reduced incorporation of polyene fatty acids into phospholipids [13]. Moreover, under conditions of oxidative stress depletion of intracellular ATP leads to acyl-CoA deficiency [2,3,13], which can result in reduced phospholipid reacylation. We previously found that palmitoylcarnitine (PC) can serve as a reserve pool of palmitoyl-CoA in erythrocytes under conditions of ATP deficiency [3].

The aim of the present study was to evaluate under conditions of oxidative stress the role of palmitic acid (resistant to oxidation) and PC in reacylation of phosphatidylethanolamine (PEA) containing primarily polyene fatty acids and constituting up to 40% of total membrane phospholipids in rat brain synaptosomes.

MATERIALS AND METHODS

Synaptosomes were isolated from rat brain [6], and protein concentration was measured using a modified method [8]. Membrane lipids were extracted according to Folch [5] and phospholipids were ana-

I. M. Sechenov Institute of Evolution Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg; *D. Annunzio University, Chieti; **Department of Pharmacology, Sigma-Tau Company, Pomezia (Rome), Italy

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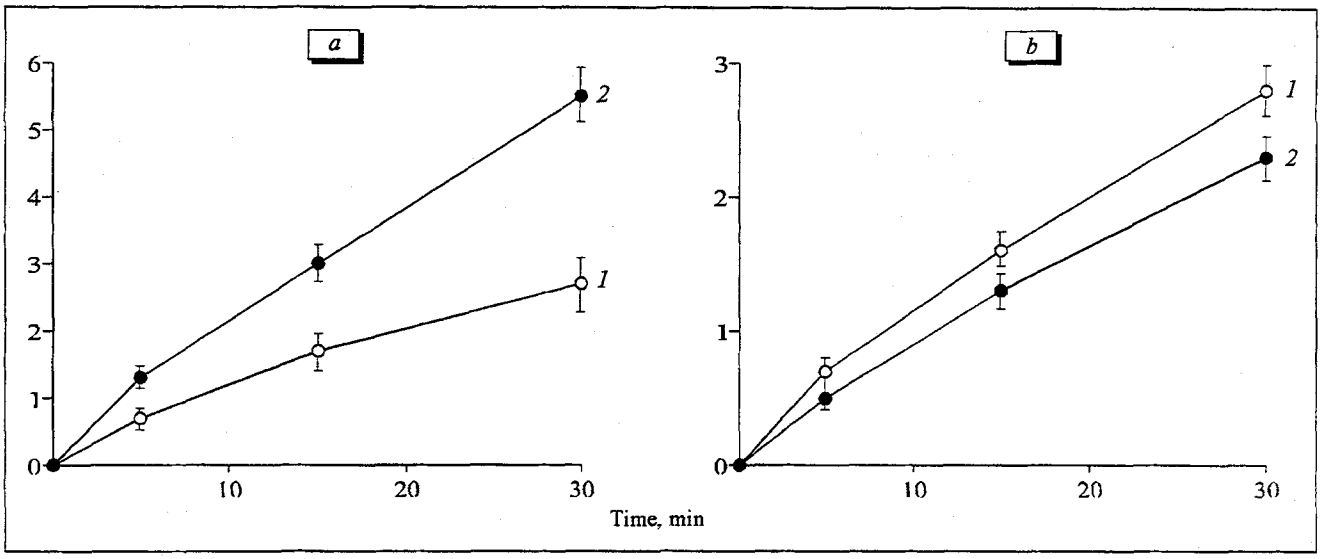

Fig. 1. Effect of LPO on incorporation of palmitic (*a*) and linoleic (*b*) acids into phosphatidylethanolamine in brain synaptosomes. *1*) control; *2*) LPO induction. Ordinate: content of [1-^{14}C]-fatty acids, pmol/μg lipid phosphorus.

lyzed by thin layer chromatography as described previously [3]. Lipid peroxidation in synaptosomes was induced using the Fe^{2+}-ascorbate system (10 μM+500 μM). The reaction was induced by adding 4-methyl-2,6-ditertbutyl phenol (500 μM). The accumulation of primary LPO products (hydroperoxides) was measured spectrophotometrically [4]. Lipid phosphorus was determined as described elsewhere [3]. Deacylation was assessed by the loss of labeled fatty acid incorporated into the second position of PEA [3]. Reacylation was evaluated by incorporation of [1-^{14}C]palmitic acid (57 mCi/mmol) and [1-^{14}C]linoleic acid (50 mCi/mmol) in a complex with bovine serum albumin (1.65 mg/ml) [3] and by incorporation of labeled palmitic acid derived from [1-^{14}C]palmitoyl-L-carnitine (58 mCi/mmol). The incubation medium contained (in mM): 40 Tris-HCl, 135 $NaCl_2$, 5 KCl, 2 $MgSO_4$, 1 NaH_2PO_4, 1 $CaCl_2$, 10 glucose, 0.02 CoA, and 1 ATP, pH 7.4. The concentration of fatty acids and PC in the incubation medium was 10 μM. Activities of acyl-CoA synthetase and acyl-CoA hydrolase were measured as described previously [13]. Activities of lysophospholipid acyltransferase and carnitine palmitoyltransferase were determined using [1-^{14}C]palmitoyl-CoA (0.5 mCi/mmol) as described elsewhere [2].

RESULTS

After induction of LPO in brain synaptosomes in the Fe^{2+}-ascorbate system the generation of alkyl (R·), oxyalkyl (RO·) and alkyl peroxide (ROO·) radicals led to accumulation of primary LPO products (fatty acid hydroperoxides (ROOH) (Table 1). Acting as Ca^{2+} iontophores, they activate endogenous Ca^{2+}-dependent phospholipases A_2 [1,7,9]. As seen from Table 1, induction of LPO markedly accelerates PEA deacylation, the rate of linoleic acid hydrolysis being

TABLE 1. Effect of LPO Induction on the Activity of Enzymes Involved into PEA Deacylation-Reacylation

Parameter	Control	LPO induction (15 min)
LPO products, nmol ROOH/mg phospholipids	8.82±0.48	36.76±2.64
Rate of PEA deacylation by endogenous phospholipase A_2, %		
[1-^{14}C]18:2*	11.00±0.55	41.48±2.05
[1-^{14}C]16:0**	2.01±0.12	25.61±1.54
Palmitoyl-CoA synthetase, nmol [1-^{14}C]16:0-CoA/mg protein/min	2.98±0.18	2.02±0.15
Palmitoyl-CoA hydrolase, nmol free [1-^{14}C]16:0/mg protein/min	2.61±0.06	2.23±0.18
LysoPEApalmitoyltransferase, pmol [1-^{14}C]16:0/mg protein/min	21.05±1.26	29.24±1.93
Carnitine palmitoyltransferase, nmol [1-^{14}C]16:0-carnitine/mg protein/min	3.80±0.23	4.30±0.26

Note. *[1-^{14}C]18:2 and **[1-^{14}C]16:0 fatty acids were preliminary incorporated at the second position of PEA molecule; initial incorporation was 3.41 and 5.55 nmol/μg lipid phosphorus, respectively.

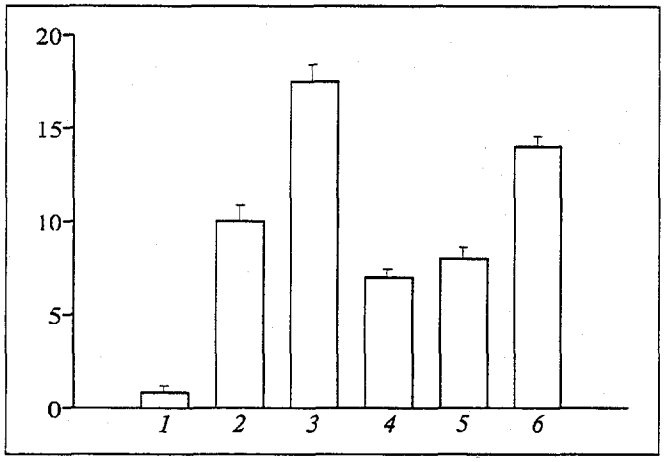

Fig. 2. Effect of LPO and carnitine palmitoyltransferase on incorporation of [1-^{14}C]-palmitoylcarnitine (PC)-derived [1-^{14}C]-palmitate into phosphatidylethanolamine in brain synaptosomes in the absence of ATP (15 min after LPO induction). Ordinate: content of [1-^{14}C]-palmitate, pmol/μg lipid phosphorus. *1*) synaptosomes, control, [1-^{14}C]-palmitate; *2*) synaptosomes, control, [1-^{14}C]-PC; *3*) synaptosomes, LPO induction, [1-^{14}C]-PC; *4*) synaptosomes+malonyl-CoA (20 μM), [1-^{14}C]-PC; *5*) synaptosomes+tetradecyl glycinate (5 μM), [1-^{14}C]-PC; *6*) synaptosomes+rotenone (17 μg/ml), [1-^{14}C]-PC.

higher than that of palmitic acid. When studying PEA reacylation under conditions of LPO induction, it was found that incorporation of palmitic acid increased while that of linoleic acid decreased in comparison with the control (Fig. 1, *b*). This agrees with previous report demonstrating very low incorporation of linoleic acid hydroperoxide into phospholipids [13]. The reduced rate of incorporation of linoleic acid into PEA in oxidative stress results from a high rate of deacylation of linoleate-containing PEA (Table 1) and inhibition of linoleoyl-CoA synthetase and lysophosphatidylethanolamine linoleoyltransferase (data not shown). It should be noted that LPO induction in synaptosomes resulted in inhibition of palmitoyl-CoA synthetase, palmitoyl-CoA hydrolase, and activation of lysophosphatidylethanolamine palmitoyltransferase (Table 1). A question arises, what is the cause of enhanced incorporation of palmitic acid into PEA against the background of high activity of palmitoyl-CoA hydrolase and inhibition of palmitoyl-CoA synthetase? Data in Table 1 indicate that LPO activates carnitine palmitoyltransferase catalyzing the following ATP-independent reaction: PC+CoA=palmitoyl-CoA+carnitine [2], supplying palmitoyl-CoA pool in the membrane. Indeed, in the absence of ATP incorporation of palmitic acid into PEA is very low; the use of PC as the substrate sharply rises this parameter especially in the presence of rotenone (Fig. 2). Induction of LPO in the absence of ATP also stimulated incorporation of PC-derived palmitate into PEA. Inhibition of carnitine palmitoyltransferase with malonyl-CoA or tetradecyl glycinate markedly reduced incorporation of palmitate into PEA, which confirms the involvement of this enzyme into PEA reacylation (Fig. 2).

Thus, our data suggest that the repair system in the brain synaptosome membranes acts through activation of deacylation reactions, which remove oxidized fatty acids from PEA molecules followed by their reacylation. Under conditions of oxidative stress, saturated (oxidation-resistant) palmitic acid is primarily incorporated into PEA. Moreover, carnitine palmitoyltransferase plays a crucial role in these reparative processes, while PC can serve as a reserve pool of activated fatty acids.

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