Phospholipid Spectrum and Lipid Peroxidation in Cell Membranes of Brain Tissue in Thymectomized Rats

V. Yu. Serebrov and O. A. Timin

UDC 616.438-089.87:612.82

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 7, pp. 22-24, July, 1994
Original article submitted November 16, 1993

Lipid peroxidation (LPO) processes and the phospholipid (PL) content and composition in the homogenate and in the mitochondrial and supramitochondrial compartments of brain cells of adult male rats were studied 3 and 6 months after thymectomy. Thymectomy was found to markedly increase the rate of LPO and to reduce antiradical activity in all cellular compartments. A reliable increase of the level of total phospholipids in the homogenate and mitochondria was observed by the third month after thymectomy and a reduction of its control levels during the sixth month postoperation. On the other hand, a reduction of the content of total phospholipids was observed in the supramitochondrial fraction by the sixth month of the experiment. An increase of the phosphatidylethanolamine (PE) and phosphatidylserine (PS) contents was found to be mainly responsible for the changes in the level of total phospholipids.

Key Words: *thymectomy; phospholipids; lipid peroxidation*

The thymus is known to secrete hormonal factors contributing to metabolism regulation [9]. The reaction of cellular systems to stimuli of different nature depends on the quantitative and qualitative composition of the phospholipids of plasma and intracellular membranes. Since LPO is known as one of the factors determining the content and composition of phospholipids [7], a study of these processes in thymectomized animals is of special interest.

The aim of this research was to examine the phospholipid spectrum and LPO processes in brain tissue during induced insufficiency of thymic function.

MATERIALS AND METHODS

Experiments were carried out with 60 male white rats weighing 150 to 180 g. The thymus was removed as described previously [2] under ether anesthesia. The studies were carried out 3 and 6 months after the operation. The animals were decapitated under light ether narcosis, after which the brain was immediately removed. Subsequent stages were carried out at 0-4°C. Subcellular fractions for measurement of total phospholipids were isolated by differential centrifugation [14]. The isolation medium contained 0.25 mol sucrose solution with 1 mmol EDTA and 0.2 mol Tris-HCl buffer (pH 7.4). Lipid extraction was carried out as described previously [12]. The content of total phospholipids was assessed from the reaction with ferrothiocyanate reagent [6]. Phospholipid fractions were separated by thin-layer chromatography on Silufol-UV-246 plates in a mixture of solvents, chloroform:methanol:water (65:25:4) [3]. Chromatograms were quantitatively assessed by elution of individual fractions in a chloroform:ethanol (2:1) mixture after their development by spraying a 2% alcohol solution of phosphomolybdic acid and subsequent measurement of optical density at 620 nm. The final results were estimated in rela-

Department of Biochemistry, Biomedical Faculty, Siberian Medical University, Tomsk. (Presented by E. D. Gol'dberg, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0007-0701$12.50 ©1995 Plenum Publishing Corporation

TABLE 1. Level of Total PL (mg/g tissue), LPO Rate (nM Malonic Dialdehyde/min/mg Protein), and Level of Antiradical Activity (nM Hydroquinone/g Lipids) ($M \pm m$)

Animals		Parameter	Fraction		
			homogenate	supramitochondrial	mitochondrial
3 months after thymectomy	Control	Total PL	68.10±10.20	11.28±1.67	1.04±0.16
		LPO rate	23.51±3.10	112.54±31.57	59.92±9.93
		Antiradical activity		16.10±0.88	25.60±7.15
	Experiment	Total PL	100.50±8.85*	9.29±3.19	1.63±0.30*
		LPO rate	88.56±10.52*	206.78±20.61*	139.26±21.84*
		Antiradical activity		2.81±0.21*	0.75±0.21*
6 months after thymectomy	Control	Total PL	65.71±6.48	10.40±1.35	1.08±0.52
		LPO rate	22.80±0.92	155.17±28.34	60.47±10.81
		Antiradical activity		10.50±3.53	26.40±2.12
	Experiment	Total PL	61.85±9.29	5.61±0.59*	1.08±0.10
		LPO rate	56.30±6.74*	608.58±46.73*	423.64±54.38*
		Antiradical activity		2.82±0.12*	1.78±0.08*

Note. Here and in Table 2: asterisk shows $p \leq 0.05$.

tive units. The LPO rate was assessed from the kinetics of formation of the secondary product, malonic dialdehyde [1], antiradical activity was estimated using diphenyl-α-picrinhydrazine [13], and protein content was measured by the biuret method [4]. The data were statistically processed using the nonparametric Wilcoxon-Mann-Whitney test [8].

RESULTS

Analysis of the results indicates that removal of the thymus leads 3 and 6 months later to a considerable increase of the LPO rate and to a reduction of antiradical activity in the fractions of cellular centrifugate of the brain (Table 1). The changes of total phospholipids in various tissue compartments are not so clear-cut (Table 1). By the third month of the experiment total phospholipids accumulate in the homogenate and mitochondria, while during the sixth month their content returns to the baseline levels. In contrast to this, in the supramitochondrial fraction of brain tissue the level of total phospholipids in thymectomized animals drops by the sixth month.

For a more detailed understanding of the changes which had taken place we examined the qualitative composition of phospholipids of various cellular compartments (Table 2). The most manifest shifts in the phospholipid spectrum of thymectomized animals are observed 3 months after the beginning of the experiment, involving the fractions

TABLE 2. Content of Total PL Fractions (in rel.%) in the Homogenate and Supramitochondrial and Mitochondrial Fractions of Cellular Centrifugate of Brain Tissue of Thymectomized Rats ($M \pm m$)

Animals	Fraction of cellular centrifugate	Content of PL fraction					
		LPC	SM	PC	PI	PE+PS	PGP+PA
		3 months after thymectomy					
Control	Homogenate	3.94±1.89	4.57±0.52	31.00±1.58	10.28±1.52	44.24±1.68	7.60±1.62
	Supramitochondrial	1.48±0.31	5.14±0.78	25.36±1.70	17.30±1.34	49.37±2.65	3.06±1.16
	Mitochondrial	1.34±0.67	6.53±1.24	21.07±2.16	9.51±1.82	51.04±3.82	11.70±2.07
Experiment	Homogenate	0.40±0.05*	5.62±1.36	24.86±1.34	4.75±0.18*	54.73±2.04*	8.17±3.03
	Supramitochondrial	2.55±0.23	2.56±0.63	25.08±1.91	9.44±2.17*	45.86±9.05	3.01±0.34
	Mitochondrial	1.20±0.82	4.49±1.49	23.43±0.47	9.67±2.14	51.75±3.15	6.39±0.63
		6 months after thymectomy					
Control	Homogenate	4.11±0.38	4.89±0.62	30.26±1.91	10.82±1.41	45.23±1.75	8.15±0.46
	Supramitochondrial	0.97±0.13	6.02±0.37	24.58±1.06	17.94±1.45	48.45±1.97	2.13±0.22
	Mitochondrial	1.16±0.11	4.21±0.76	20.63±1.35	9.13±0.25	50.51±1.86	12.36±1.23
Experiment	Homogenate	0.20±0.03*	5.94±2.30	29.07±3.22	12.54±1.57	43.02±6.22	10.64±2.75
	Supramitochondrial	2.83±0.38	3.28±1.01	23.72±2.17	12.58±2.72	44.54±2.54	4.31±1.20
	Mitochondrial	2.61±1.50	6.33±1.46	28.26±3.34	5.22±0.82*	47.98±5.35	13.43±7.06

Note. SM – sphingomyelin; other abbreviations as in the text.

of PE+PS, phosphatidylinositides (PI), and lysophosphatidylcholine (LPC) in the extramitochondrial part of the cells. Accumulation of the PE+PS fraction is mainly responsible for the changes in the total phospholipid level, the share of this fraction being the highest among all the phospholipid fractions. Thymectomy may alter the activity of the enzymes contributing to aminophosphatide metabolism. The selective reduction of phospholipids in the supramitochondrial fraction may be due to the presence of two phospholipid pools in the endoplasmic reticulum: the pool proper and the exported pool [10], which have different regulation systems.

The metabolism of PI is closely related to the effects of hormones and neurotransmitters capable of inducing the accumulation of Ca^{2+} ions in the cell [5], this being a potent activator of PI-specific phospholipase C [11]. The effect of PI reduction in membranes may be indicative of intensive stimulation of receptors whose activation induces an increase of the Ca^{2+} level in the cytosol. The absence of thymic hormones has been shown to lead to the accumulation in the brain tissue of Ca^{2+} agonists: catecholamines, serotonin, and vasopressin [9].

A drop of the LPC level may be caused by decreased activity of phospholipase A_2 (PLA_2), although a reduction of the phosphatidylcholine (PC) level by 30% indicates another, more probable cause: PLA_1 and PLA_2 activation leads to the utilization of PC to free fatty acids and deacylated residue. Unsaturated fatty acids are the LPO substrate, while saturated ones may be utilized in the synthesis of PE and PS. A marked reduction of polyglycerophosphatides (PGP) and phosphatidic acid (PA) in the mitochondria indicates activation of mitochondrial Ca^{2+}-dependent PLA_2 selectively hydrolyzing cardiolipin [15].

Hence, the changes in the spectrum of membrane phospholipids and LPO processes attest to the sensitivity of brain tissue to a deficiency of thymic hormones. Increases in the LPO rate and phospholipid content may have an effect on the function of brain structures and impair brain activity.

REFERENCES

1. Yu. A. Vladimirov and A. I. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972).
2. S. G. Gasanov and T. S. Yakusheva, *Probl. Endokrinol.*, № 2, 29 (1961).
3. O. N. Zamuruev, *Byull. Eksp. Biol. Med.*, **86**, № 10, 419 (1978).
4. G. A. Kochetov, in: *Handbook of Enzymology* [in Russian], Moscow (1980).
5. N. E. Kucherenko and Ya. B. Blyum, *Ukr. Biokhim. Zh.*, **58**, № 1, 29 (1986).
6. A. A. Pentyuk, V. I. Gutsol, and N. B. Yakovleva, *Lab. Delo*, № 6, 457 (1987).
7. Yu. A. Petrovich and D. V. Gutkin, *Pat. Fiziol.*, № 5, 10 (1986).
8. I. V. Polyakov and N. S. Sokolova, *Handbook of Medical Statistics* [in Russian], Leningrad (1975).
9. V. Yu. Serebrov and S. L. Stukanov, *The Endocrine Function of the Thymus* [in Russian], Tomsk (1993).
10. D. A. Chetverikov, in: *Biochemistry of Lipids and their Role in Metabolism* [in Russian], Moscow (1981), p. 155.
11. J. M. Augustyn and W. B. Elliot, *Ann. Biochem.*, **31**, 246 (1969).
12. J. Folch, M. Less, and G. Sloan-Stanley, *J. Biol. Chem.*, **226**, 497-509 (1957).
13. J. Glavind, *Acta Biochem. Scand.*, **17**, № 6, 1655-1659 (1963).
14. D. Jonson and H. Lardy, *Methods of Enzymology*, **25**, 56-64 (1967).
15. P. M. Vignais, J. Nachbaur, P. V. Vignais, and J. Andre, *Mitochondria: Struct., Funct., 5th Fed. Eur. Biochem. Soc. Meet.*, p. 43.