4. N. Ya. Kovalenko and D. D. Matsievskii, *Byull. Eksp. Biol. Med.*, **114**, No. 8, 128-130 (1992).
5. M. A. Ponomareva-Stepnaya, V. N. Nezavibat'ko, L. V. Antonova, *et al.*, *Khim.-Farm. Zh.*, No. 7, 790-795 (1984).
6. K. V. Sudakov, *Usp. Fiziol. Nauk*, **25**, No. 4, 79 (1994).
7. L. S. Ul'yaninskii, M. A. Zvyagintseva, and I. L. Kosharskaya, *Byull. Eksp. Biol. Med.*, **109**, No. 5, 419-420 (1990).
8. V. K. Khugaeva, *Ibid.*, **112**, No. 8, 117-120 (1991).
9. V. K. Khugaeva, *Fiziol. Zh. SSSR*, **7**, No. 12, 109-118 (1992).
10. K.-J. Chang and P. Cuatrecasas, *Fed. Proc.*, **40**, 2729-2734 (1984).
11. J. Gasparik, *Eur. J. Pharmacol.*, **172**, No. 6, 417-424 (1990).
12. J. Irgensen, *Peptides*, **12**, No. 4, 831-837 (1991).

Phospholipid Composition of Liver Mitochondria in Experimental Hemorrhagic Shock

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Heparin used as an anticoagulant in modeled hemorrhagic shock decreases the phosphatidylcholine and increases the phosphatidylethanolamine contents in the mitochondria. Accumulation of lysophosphatidylcholine in whole mitochondria and their inner membrane is observed in hemorrhagic shock. At the same time, hemorrhagic shock decreases phosphatidylcholine content in the inner and outer mitochondrial membranes and increases phosphatidylethanolamine content in the outer membranes. Modification of phospholipid composition of mitochondrial membranes is a mechanism responsible for impaired energy production in liver mitochondria in hemorrhagic shock.

Key Words: *phospholipids; mitochondria; liver, hemorrhagic shock*

Energy deficiency, which limits tissue functions, is a cause of shock irreversibility. Under normal conditions 95% of energy in the body is produced by the mitochondria [7]. In shock, energy production in liver mitochondria progressively impairs [9], and the mechanisms of this process remains unclear. There is evidence that alterations of phospholipid turnover in the mitochondria may reduce their functional activity [5,8,11]. It should be noted that in shock the liver is the first organ losing the energy-producing function [9].

In an attempt to elucidate the mechanisms of modification of energy production in the mitochondria and to find out the ways to correct these modifications, we decided to study changes in the phospholipid composition of liver mitochondrial membranes in experimental hemorrhagic shock. Since high doses of heparin, which is known to modify lipid metabolism, have been used to prevent blood coagulation in catheters, we examined the effect of heparin in the phospholipids of liver mitochondria.

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MATERIALS AND METHODS

Experiments were performed on 15 cats (body weight 3.0±0.5 kg) under Nembutal anesthesia (40 mg/kg intraperitoneally). Hemorrhagic shock was produced as described elsewhere [14]. For prevention of blood coagulation in catheters heparin was injected in a dose of 2000 U/kg. Blood was drained 30 min after the injection until blood pressure dropped to 40 mm Hg. Blood pressure was maintained at this level for 1 h. The cats were sacrificed after 1.5 h of blood loss. Intact animals injected and not injected with heparin served as controls. Material for the investigation was obtained 2 h after anesthesia. Liver mitochondria and their inner and outer membranes were

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isolated by the method [10] in medium containing 0.25 M sucrose, 5 mM Tris-HCl (pH 7.4), and 0.5 mM EDTA. After extraction of total lipids from mitochondria and their membranes [12], phospholipids were fractionated by one-dimension thin-layer chromatography on Silufol UV-254 plates (Cavalier) using the following solvent systems: acetone:petroleum ether (1:3) and chloroform:methanol:acetic acid:water (32:5.2:7.2:0.12) [13]. Chromatograms were analyzed with the use of a Chromoscan-201 densitometer (Joyce-Loebl) and semiautomatic image analyzer (Leitz-A.S.M.). The data were processed by variational statistics methods.

RESULTS

Changes in the phospholipid compositions of whole mitochondria were observed 2 h after heparin injection. The phosphatidylethanolamine (PE) content increased by 50.4% ($p<0.001$), while the phosphatidylcholine (PC) content decreased by 26.5% ($p<0.01$, Table 1). Heparin had no effect on the phospholipid composition of mitochondrial membranes.

During hemorrhagic shock the lysophosphatidylcholine (LPC) content in whole mitochondria increased 2.5-fold ($p<0.05$), and considerable changes were observed in the phospholipid spectrum of mitochondrial membranes, particularly in the outer membranes. The content of PE increased by 25.1% ($p<0.01$), PC content decreased by 30.6% ($p<0.01$), and LPC content increased 2.6-fold ($p<0.01$). The phospholipid spectrum of inner membranes also changed: the PC content decreased by 22.1% ($p<0.05$).

Thus, heparin injected in the dose preventing blood coagulation in experimental hemorrhagic shock had no effect on phospholipid composition of mitochondrial membranes and modifies PC and PE contents of whole mitochondria. These findings suggest that heparin-induced changes in the mitochondria occur at the contacts between inner and outer mitochondrial membranes. The density of these sites is intermediate between that of inner and outer membranes [4]. We did not investigate these sites in this study. It is known that the contacts between inner and outer mitochondrial membranes are the transport zones of *de novo* synthesized phospholipids between mitochondria and intracellular organelles [4]. Consequently, heparin-induced changes in mitochondria may reflect modifications in the intracellular metabolism of phospholipids, which do not occur in mitochondrial membranes.

During hemorrhagic shock changes in the phospholipid spectrum of whole mitochondria were associated with the accumulation of LPC. This phospholipid was accumulated in the outer membrane, where phospholipids are more sensitive to damage [6]. In addition, an increase in LPC content of the outer membrane was accompanied by a decrease in PC content, which presumably testifies to the activation of phospholipases and insufficient rate of LPC reacylation. Bearing in mind that LPC loosens the membrane lipid bilayer and increases membrane permeability, it can be suggested that LPC accumulation is a factor of the mitochondrial membrane damage in hemorrhagic shock.

It should be stressed that PC content decreased both in inner and outer mitochondrial membranes.

TABLE 1. Phospholipid Composition of Liver Mitochondria and Their Membranes (%) in Cats Under Normal Conditions and Hemorrhagic Shock ($M\pm m$)

Group (n=5)	Cardiolipin	Phosphatidyl-ethanolamine	Phospha-tidylserine	Phospha-tidylinositol	Phospha-tidylcholine	Sphingo-myelin	Lysophospha-tidylcholine
Mitochondria							
Background	10.2±1.9	27.4±1.9	4.7±1.5	4.6±1.2	45.6±2.9	6.3±1.5	1.4±0.3
Control	13.4±3.3	41.2±1.4*	3.7±0.6	4.1±1.5	33.5±1.6*	3.4±0.8	0.8±0.1
Shock	16.8±3.3	36.1±3.5	3.4±0.6	3.5±1.2	33.2±3.6	4.6±0.9	2.0±0.5**
Outer membranes							
Background	—	46.1±2.9	4.5±0.7	6.7±1.3	38.1±4.8	4.1±1.5	1.5±0.6
Control	—	42.2±2.1	4.8±0.5	4.2±0.4	41.5±2.9	5.3±1.8	1.3±0.4
Shock	—	52.8±1.9**	6.9±1.2	4.7±0.9	28.8±1.8**	4.0±1.0	3.4±0.4**
Inner membranes							
Background	9.8±2.4	39.4±2.8	4.2±1.2	3.6±1.8	36.2±1.9	3.5±0.7	3.2±0.8
Control	11.2±2.8	34.8±4.6	5.3±1.5	4.1±0.9	38.9±2.2	3.4±0.9	2.0±0.5
Shock	12.1±3.7	38.9±4.3	4.9±0.6	3.0±0.5	30.3±2.3**	4.3±1.3	6.5±2.6

Note. $p<0.05$: *compared with background; **compared with the control.

Since PC play a specific regulatory role in lipid peroxidation in the cell membranes [1-3], it can be hypothesized that the decrease in the PC content of mitochondrial membranes is one of the mechanism by which the mitochondrial antioxidant protection is reduced in hemorrhagic shock.

An increase in the PE content of the outer mitochondrial membranes is an important factor acting in hemorrhagic shock. It should be noted that PE has a high content of polyunsaturated fatty-acid acyls, therefore, its accumulation in the mitochondria during shock may be a prerequisite of activation of lipid peroxidation. In addition, PE regulates membrane permeability. It was found that increased PE content is associated with high activity of calcium pump [15]. Based on these findings, it can be suggested that PE accumulation in the outer membranes of liver mitochondria in hemorrhagic shock contributes to the increase in their permeability.

Thus, our results indicate that heparin used in the dose preventing blood coagulation in modeled hemorrhagic shock changes the contents of PE and PC, the major phospholipids of liver mitochondria. Hemorrhagic shock is accompanied by changes in the phospholipid spectrum of mitochondrial membranes (decrease in PC and increase in PE and LPC contents) and increased risk of lipid peroxidation in the mitochondria. Thus, modification of phospholipid composition of inner and outer mitochondrial membranes can be regarded as a cause of impaired energy production during hemorrhagic shock. Reparation of mitochondrial membranes should be taken into consideration when new methods of hemorrhagic shock management are developed.

REFERENCES

1. E. B. Burlakova, A. V. Alekseenko, S. A. Aristarkhova, *et al.*, in: *Lipids of Biological Membranes* [in Russian], Tashkent (1982), pp. 16-23.
2. E. B. Burlakova, S. A. Aristarkhova, L. V. Fedorova, *et al.*, *Biol. Nauki*, No. 9, 21-27 (1991).
3. Yu. M. Petrusevich, G. V. Kossova, and O. R. Kol's, in: *Superweak Luminescence in Biology* [in Russian], Moscow (1972), pp. 133-136.
4. D. Ardail, J.-P. Privat, M. Egret-Charlier, *et al.*, *J. Biol. Chem.*, **265**, 18797-18802 (1990).
5. M. C. Beatrice and S. Socudi, *Biochem. Soc. Trans.*, **13**, 732-733 (1985).
6. M. C. Block, K. W. Virtz, and G. L. Scherphof, *Biochim. Biophys. Acta*, **233**, 61-75 (1971).
7. J. H. Chaudry, *Am. J. Physiol.*, **245**, R117-R134 (1983).
8. K. R. Chien, J. Abrame, A. Sevroni, *et al.*, *J. Biol. Chem.*, **253**, 4809-4817 (1978).
9. R. R. Kirby, *Int. Anesthesiol. Clin.*, **25**, 19-35 (1987).
10. M. Levy, R. Toury, and J. Andre, *Biochim. Biophys. Acta*, **135**, 599-613 (1962).
11. N. Masaki, N. Kyle, A. Seeroni, and J. L. Farber, *Arch. Biochem. Biophys.*, **270**, 672-680 (1989).
12. A. Rethy, V. Tomasi, and A. Trevisani, *Ibid.*, **147**, 36-40 (1971).
13. V. P. Skipsky, V. Barklay, E. S. Reichman, and J. J. Good, *Biochim. Biophys. Acta*, **137**, 80-89 (1967).
14. C. T. Wiggers, *Physiology of Shock*, London (1950).
15. P. L. Yeagle, *FASEB J.*, **3**, 1833-1842 (1989).