Changes in Phospholipid Composition of Mitochondria in the Medulla Oblongata and Frontal Lobes of the Cerebral Hemispheres in Hemorrhagic Shock in Cats

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Considerable regional differences in the phospholipid composition of mitochondrial membranes are found in the brain of cats in the terminal phase of hemorrhagic shock. The most prominent alteration is noted in the medulla oblongata and consists in a progressive elimination of phosphatidylcholine. Changes in the main phospholipids in mitochondrial membranes of the cerebral hemispheres are less pronounced and consist in a drop of phosphatidylinositol and phosphatidylethanolamine. Accumulation of lysophosphatidylcholine and lysophosphatidylethanolamine is a regular feature of the studied mitochondria. Accumulation of lysophosphatidylserine is found primarily in mitochondrial membranes of the medulla oblongata.

Key Words: *phospholipids; mitochondria; brain; hemorrhagic shock*

Cell functional activity is known to depend strictly on the synthesis and utilization of ATP [14,16,18]. Under aerobic conditions about 80% of the ATP in the brain is generated in the course of mitochondrial oxidative phosphorylation, whereas in anoxia or total cerebral ischemia the ATP reserves may be depleted within a few minutes [16]. Disturbances in phospholipid metabolism accompanied by accumulation of products of their hydrolysis are considered to be among the factors responsible for disturbances in energy production in the mitochondria [2,14]. In light of the above, we studied the phospholipid composition of mitochondria and their membranes isolated from different areas of the brain in modeled hemorrhagic shock.

MATERIALS AND METHODS

The experiments were carried out on 17 cats weighing 3.0±0.5 kg narcotized with Nembutal (40 mg/kg intraperitoneally). Hemorrhagic shock was modeled as described previously [19]. The animals were injected with heparin in a dose of 2000 U/kg to prevent blood coagulation in the catheters and 30 minutes later the blood pressure was adjusted to 40 mm Hg through bloodletting and maintained at this level for one hour. The animals were sacrificed 1.5 hours after the onset of the blood loss. Intact animals injected with the same dose of heparin served as controls. A comparison was also made with intact cats not injected with heparin (background). Tissue samples were obtained 2 hours after narcotization. Mitochondria were isolated from the medulla oblongata and frontal lobes of the cerebral hemispheres as described previously [13] in a medium containing 0.32 M sucrose, 10 mM Tris-HCl (pH 7.4) and 1 mM EDTA [11]. The outer and inner mitochondrial membranes were isolated after Levy [12]. After extraction of total lipids from mitochondria and their membranes [15], phospholipids were separated by thin-layer chromatography on Silufol UV-254 plates (Avalier) in a system containing chloroform:methanol:7 N ammonium (12.4:4.6:1.0 v/v ratio) [4]. The chromatograms were scanned

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on a Chromoscan 201 (Joyce-Loebl) and analyzed using a semiautomatic image analyzer (Leitz-A.S.M.). The data were processed using the ANOVA Student test.

RESULTS

The dose of heparin used for modeling hemorrhagic shock was found to affect the phospholipid composition of the brain mitochondria, the content of phosphatidylinositol (PI) in the frontal lobes being increased 2.3-fold ($p<0.01$, Table 1). Moreover, heparin induced a redistribution of phospholipids in the mitochondrial membranes. In particular, PI in the outer mitochondrial membranes from the frontal lobes rose 2.2-fold in comparison with the control value ($p<0.01$), while lysophosphatidylcholine (LPC) dropped in both the inner (2.4-fold, $p<0.01$) and outer (by 43.6%, $p<0.01$) membranes. A drop of phosphatidylserine in the outer membranes was a common phenomenon for mitochondria from the frontal lobes and medulla oblongata (by 39.3 and 38.7%, respectively, $p<0.05$). In the former this drop was accompanied by a 2-fold increase of lysophos-

TABLE 1. Phospholipid Content of Mitochondria from the Medulla Oblongata and Their Membranes (in %) in Cats in Health and in Hemorrhagic Shock ($M\pm m$)

Phospholipids	Background (n=5)	Control (n=6)	Shock (n=5)
Mitochondria			
Phosphatidic acid	4.5±0.8	5.1±1.1	9.4±3.5
Cardiolipin	5.6±2.5	5.7±1.0	8.1±1.8
Phosphatidylethanolamine	31.9±4.1	27.6±3.5	34.8±7.8
Phosphatidylcholine	35.9±7.3	32.9±4.1	17.6±5.1**
Phosphatidylinositol	9.9±2.8	17.2±1.8	11.2±3.4
Sphingomyelin	5.3±2.3	3.6±0.7	4.0±1.4
Lysophatidylethanolamine	1.0±0.6	2.1±0.5	4.8±0.8**
Phosphatidylserine	2.0±0.8	3.2±0.6	4.9±1.2
Lysophosphatidylcholine	1.5±0.7	1.5±0.3	6.1±2.8
Lysophosphatidylserine	1.6±0.8	0.7±0.2	3.2±0.9**
Outer membranes			
Phosphatidic acid	10.7±1.9	7.4±2.1	7.5±2.6
Cardiolipin	-	-	-
Phosphatidylethanolamine	23.0±7.4	28.8±3.3	26.9±2.6
Phosphatidylcholine	33.0±4.7	38.6±4.1	17.4±1.2**
Phosphatidylinositol	10.5±1.4	11.4±1.8	13.1±2.1
Sphingomyelin	5.4±1.0	5.8±1.0	6.6±1.8
Lysophosphatidylethanolamine	5.0±1.0	2.4±0.8	6.4±1.8
Phosphatidylserine	6.2±0.8	3.8±0.4*	6.4±1.1
Lysophosphatidylcholine	3.9±0.8	2.1±0.6	11.0±3.1**
Lysophosphatidylserine	2.2±0.5	1.3±0.3	5.2±1.4**
Inner membranes			
Phosphatidic acid	8.0±1.7	5.0±1.2	13.1±2.4**
Cardiolipin	5.8±0.3	3.0±0.5*	4.9±1.3
Phosphatidylethanolamine	25.5±2.2	33.8±4.4	28.6±1.4
Phosphatidylcholine	33.8±4.0	26.1±4.8	11.3±1.1**
Phosphatidylinositol	6.9±0.9	13.5±3.0	11.6±2.0
Sphingomyelin	4.2±1.1	3.0±0.8	5.8±1.1
Lysophosphatidylethanolamine	4.0±0.7	3.0±0.6	6.6±1.2**
Phosphatidylserine	8.0±1.9	7.5±2.1	6.1±1.2
Lysophosphatidylcholine	3.4±1.0	2.4±0.6	7.2±1.8**
Lysophosphatidylserine	1.8±0.7	2.2±0.4	4.6±0.7**

Note. Here and in Table 2: $p<0.05$: *in comparison with the background values, **in comparison with the control. n indicates the number of animals.

phatidylserine in comparison with the control level ($p<0.01$). In the inner mitochondrial membranes from the medulla oblongata the content of cardiolipin dropped by 48.3% ($p<0.01$). It should be noted that these differences in the phospholipid spectrum of whole mitochondria and their membranes may be attributed to variations in the content of phospholipids in the contact sites between the outer and inner mitochondrial membranes. These sites, which are known to handle the phospholipid transport between mitochondria and cell organelles [5], were beyond the scope of the present study.

A characteristic outcome of hemorrhagic shock was the removal of phosphatidylcholine (PC) from mitochondrial membranes in the medulla oblongata: this parameter dropped 2.2-fold in comparison with the control level ($p<0.05$-0.01, Table 2). On the other hand, these membranes were enriched with lysophospholipids. In the outer and inner mitochondrial membranes from the medulla oblongata the content of LPC increased 5.2- and 3.0-fold, respectively ($p<0.05$), and lysophosphatidylserine 4- and 2-fold, respectively ($p<0.05$-0.01). This was accompanied by an accumulation of lysophosphatidylethanolamine: in the inner mitochondrial membranes this parameter surpassed the control level 2.2-fold ($p<0.05$). Alterations in whole mitochondria from the medulla oblongata reflected the changes in the

TABLE 2. Phospholipid Content of Mitochondria from the Frontal Lobes and Their Membranes (in %) in Cats in Health and in Hemorrhagic Shock ($M\pm m$)

Phospholipids	Background (n=5)	Control (n=4)	Shock (n=6)
Mitochondria			
Phosphatidic acid	8.4±3.8	5.4±2.5	12.2±3.8
Cardiolipin	3.5±0.8	4.6±1.2	5.0±0.4
Phosphatidylethanolamine	33.4±4.8	30.0±3.1	33.8±4.4
Phosphatidylcholine	33.9±2.3	28.6±3.0	21.0±2.7
Phosphatidylinositol	7.5±1.7	17.0±1.4*	11.9±1.4**
Sphingomyelin	4.1±1.4	2.5±0.3	5.2±1.4
Lysophosphatidylethanolamine	1.2±0.4	2.3±0.9	2.7±1.5
Phosphatidylserine	3.7±0.9	5.3±1.5	3.7±1.1
Lysophosphatidylcholine	1.8±0.4	2.1±0.3	3.2±0.8
Lysophosphatidylserine	0.9±0.3	0.8±0.2	1.9±1.1
Outer membranes			
Phosphatidic acid	8.6±2.1	6.1±1.7	9.2±0.9
Cardiolipin	-	-	-
Phosphatidylethanolamine	28.8±2.8	28.8±4.3	31.2±0.6
Phosphatidylcholine	36.2±2.0	28.0±5.2	25.4±1.8
Phosphatidylinositol	9.5±1.2	21.3±2.3*	12.2±1.8**
Sphingomyelin	4.7±1.8	6.2±2.4	6.6±1.6
Lysophosphatidylethanolamine	2.1±0.8	2.2±0.5	4.1±0.5**
Phosphatidylserine	5.6±0.6	3.4±0.6*	4.9±1.3
Lysophosphatidylcholine	4.0±0.7	1.7±0.4*	5.6±0.7**
Lysophosphatidylserine	1.0±0.2	2.0±0.1*	2.8±0.4
Inner membranes			
Phosphatidic acid	7.0±1.0	7.5±1.4	5.4±2.3
Cardiolipin	4.5±0.7	6.6±1.4	4.4±1.4
Phosphatidylethanolamine	30.4±3.2	37.2±2.1	25.3±2.9**
Phosphatidylcholine	28.9±2.8	25.4±0.6	19.6±3.4
Phosphatidylinositol	9.4±1.8	8.5±1.2	10.8±2.6
Sphingomyelin	4.0±1.2	2.8±0.4	2.6±1.0
Lysophosphatidylethanolamine	3.6±1.0	3.4±0.4	4.6±1.8
Phosphatidylserine	7.1±1.2	5.0±0.1	6.9±2.6
Lysophosphatidylcholine	3.9±0.5	2.2±0.4*	13.7±3.8**
Lysophosphatidylserine	1.9±0.3	1.4±0.2	5.9±2.4

outer and inner membranes, but were less pronounced: the level of PC was decreased only 46.5% ($p<0.05$), and among the lysophospholipids only lysophosphatidylethanolamine and lysophosphatidylserine rose, by 2.3- and 4.6-fold, respectively ($p<0.02$).

In the frontal lobes of the cerebral hemispheres hemorrhagic shock reduced the content of PI in the whole mitochondria and in their outer membranes by 30% ($p<0.05$) and 42.7% ($p<0.02$), respectively. The content of phosphatidylethanolamine in the inner membranes dropped by 32% ($p<0.02$). These shifts were also accompanied by an accumulation of lysophospholipids: LPC rose in both the outer (3.3-fold, $p<0.01$) and inner (6.2-fold, $p<0.02$) mitochondrial membranes, while lysophosphatidylethanolamine primarily accumulated in the outer membranes (1.9-fold in comparison with the control level, $p<0.05$).

Thus, in hemorrhagic shock the most pronounced alteration in the phospholipid spectrum of mitochondrial membranes was observed in the medulla oblongata. These changes primarily consisted in a progressive elimination of PC from mitochondrial membranes. Since PC has been shown to act as an inhibitor of lipid peroxidation [3], our findings may attest to a severe depletion of the antioxidant defense system in mitochondria of the medulla oblongata. It should be noted that the release of choline from PC in the central nervous system is crucial for acetylcholine synthesis when the liberation of this transmitter is enhanced and, consequently, a large amount of its precursor is required [9]. Thus, the progressive elimination of PC from mitochondrial membranes of the medulla oblongata in hemorrhagic shock corroborates the previous data on the utilization of neuronal choline-containing phospholipids for acetylcholine synthesis. This results in damage to the cell membranes and leads to cell death [8]. On the other hand, the increased content of phosphatidic acid in the inner mitochondrial membranes may result from the augmented degradation of PC combined with inhibition of the subsequent conversion of phosphatidic acid into diglycerides.

In hemorrhagic shock we observed a reduced content of PI in the outer mitochondrial membranes from the frontal lobes. PI metabolites are known to play an important role in the functioning of the brain [10] by participating in the regulation of neurochemical processes related to neurotransmission [7,17]. In the course of hemorrhagic shock, PI from the outer mitochondrial membranes of cortical neurons may be used to replenish the intracellular stores of these metabolites. The elimination of phosphatidylethanolamine from the inner mitochondrial membranes of the frontal lobes should also be noted, since its level in the cell membranes affects their specific ionic conductivity [1].

Accumulation of lysophospholipids is considered to be partially responsible for increased nonspecific permeability of mitochondrial membranes [2,6]. In light of this the elevation of LPC in mitochondrial membranes of the medulla oblongata and frontal lobes merits attention. Accumulation of lysophosphatidylserine was noted primarily in mitochondrial membranes of the medulla, while lysophosphatidylethanolamine accumulated both in the inner mitochondrial membranes of the medulla and outer mitochondrial membranes of the cerebral hemispheres.

Our findings may contribute to understanding of the mechanisms underlying the reduced adaptation capacity of brain cells in developed hemorrhagic shock.

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