Changes in Phospholipid Composition of Synaptic Membranes in Frontal Lobes of Cerebral Hemispheres in Cats at Various Stages of Hemorrhagic Shock

G. F. Leskova

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Phospholipid composition of synaptic membranes in the frontal lobes of cerebral hemispheres was studied in cats with hemorrhagic shock. The compensatory and adaptive mechanisms of regulation of neurotransmission in this region of the brain at the initial stage of hemorrhagic shock are associated with increased degradation of phosphatidylinositol. Accumulation of this phospholipid in synaptic membranes during severe hemorrhagic shock reflects instability of the key neuroregulatory pathway, which is mediated by phosphatidylinositol metabolites. Dysregulation of transmembrane signaling in hemorrhagic shock is related to depletion of phosphatidylcholine and phosphatidylserine in synaptic membranes and accumulation of phosphatidylethanolamine.

Key Words: *phospholipids; synaptic membranes; hemorrhagic shock*

Structural and functional changes in the central nervous system (CNS) contribute to the pathogenesis of hemorrhagic shock (HS) and underlie the development of posthemorrhagic encephalopathies. It is important to study the compensatory response and pathological processes in the brain during massive blood loss. Brain function is determined by phospholipid (PL) metabolism in neuronal membranes. These compounds serve as potent neuromodulators. A relationship exists between variations in the composition of membrane PL and type of biological signal transmission. PL metabolites in the neuronal plasmalemma have a strong activating effect on various membrane-bound enzymes, endocytosis, and exocytosis of neurotransmitters. They regulate the pathogenetic mechanisms of several neurodegenerative diseases. Degradation of membrane PL plays an important role in neuronal death under pathological conditions [9].

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** leskovagalina@rambler.ru. G. F. Leskova

Here we studied the PL composition of synaptic membranes (SM) in the frontal lobes of cerebral hemispheres at various stages of HS. The goal of our study was to evaluate the role of metabolic changes in membrane PL in the compensatory mechanisms of neurotransmission and neurochemical dysregulation in this region of the brain.

MATERIALS AND METHODS

Experiments were performed on 29 cats weighing 2.5-3.5 kg. The animals were anesthetized with nembutal (40 mg/kg intraperitoneally). HS was produced by the method of Wiggers and Fine [4,15]. The cats received heparin in a dose of 2000 U/kg to prevent blood coagulation in catheters. The blood was withdrawn into a reservoir 30 min after heparin administration. Blood pressure was reduced to 40 mm Hg over 30 min and maintained at this level for 30 min and 1 h. The control group consisted of intact animals receiving heparin in the specified dose. The animals were euthanized with nembutal in a dose of 90 mg/kg. The frontal lobes were dis-

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sected 1, 1.5, and 2 h after heparin administration (control group) or 0.5, 1, and 1.5 h after blood loss (treatment group). SM were isolated from the frontal lobes as described elsewhere [8]. Total lipids were extracted by the method of Folch. PL were fractionated by thin-layer chromatography on Silufol UV-254 plates using a solvent system of chloroform, methanol, and 7 N ammonia (12.4:4.6:1) [1]. Chromatograms were subjected to densitometry on a Chromoscan-201 device (Joyce-Loebl). Densitograms were analyzed on a Leitz-A.S.M. semiautomatic image scanner. PL content was calculated in percent of the total peak area in densitograms. The results were analyzed by Student's *t* test.

RESULTS

The PL composition of SM from the frontal lobes of the cerebral hemispheres was modified after blood loss for 30 min. The contents of phosphatidylinositol and phosphatidylcholine decreased by 2.0 and 1.3 times, respectively, compared to the control ($p<0.01$, Table 1). The ratio of phosphatidylethanolamine increased by 1.4 times ($p<0.01$). After 1-h blood loss the concentration of phosphatidylcholine in SM remained low (1.3-fold lower compared to the control, $p<0.02$), while the content of phosphatidylethanolamine increased by 1.2 times ($p<0.01$). The ratio of phosphatidylinositol and phosphatidylethanolamine in SM increased by 1.9 and 1.1 times, respectively, after blood loss for 1.5 h ($p<0.02$-0.05). These changes were accompanied by a 2.1-fold decrease in the content of phosphatidylserine ($p<0.02$). It should be emphasized that the content of PL in SM from control animals differed in the early and late stages of the study. Phosphatidylethanolamine content 1.5 h postinjection was lower than after 0.5 and 1 h (by 1.3 and 1.5 times, respectively, $p<0.010$-0.001). Phosphatidylinositol content 1 and 1.5 h postinjection was 2-fold lower than after 0.5 h ($p<0.02$-0.01). Sphingomyelin content 1.5 h postinjection was 4 times higher than that after 0.5 and 1 h ($p<0.02$-0.01). The contents of lysophosphatidylethanolamine and phosphatidylserine 1.5 h postinjection were 4-fold higher than those after 0.5 h ($p<0.02$-0.01). These differences were probably related to the effect of heparin. Heparin in the specified doses has a strong effect on lipid metabolism under conditions of experimental HS.

Our results indicate that variations in the PL composition of SM form the frontal lobes of the cerebral hemispheres during the initial stage of HS manifested in a decrease in phosphatidylinositol content. Phosphatidylinositol metabolites are involved in the mechanisms of transmembrane signaling. Depletion of phosphatidylinositol in SM is probably induced by adrenergic and cholinergic activation. This state is associated with blood loss and results in the expression of phosphatidylinositol-sensitive phospholipase C [14]. Phosphatidylinositol derivatives (phosphoinositides) play a key role in stimulation of neurotransmission. It may be suggested that the increased production of these compounds plays an important role in the maintenance of adrenergic and cholinergic hyperactivation at the initial stage of HS. Moreover, phosphoinositides contribute to the increase in Ca^{2+}-depen-

TABLE 1. Phospholipid Composition of SM from Frontal Lobes of Cerebral Hemispheres in Cats (%, *M*±*m*)

Parameter	Period, h					
	0.5		1		1.5	
	control (*n*=4)	treatment (*n*=5)	control (*n*=5)	treatment (*n*=4)	control (*n*=5)	treatment (*n*=6)
Phosphatidic acid	1.9±0.8	1.9±0.7	2.1±1.0	4.1±1.4	3.5±1.0	3.5±0.8
Phosphatidylethanolamine	36.0±1.8	49.0±1.7*	41.4±1.9^{+}	51.0±1.0*	27.6±1.1^{+x}	31.3±1.1*
Phosphatidylcholine	36.1±0.8	27.1±1.6*	36.5±2.3	28.3±1.0*	31.8±4.9	28.9±2.4
Phosphatidylinositol	18.7±1.7	9.2±0.9*	10.0±1.9^{+}	10.5±1.6	9.3±1.3^{+}	17.6±2.2*
Sphingomyelin	3.8±1.4	6.4±0.9	3.9±1.9	2.4±0.9	14.6±2.7^{+x}	12.6±3.7
Lysophosphatidylethanolamine	0.7±0.3	0.9±0.1	1.8±0.6	1.5±0.7	2.8±0.7^{+}	2.6±0.2
Phosphatidylserine	1.2±0.3	3.1±0.9	2.4±0.7	1.5±0.4	5.0±0.8^{+}	2.4±0.5*
Lysophosphatidylcholine	0.6±0.3	1.7±0.7	1.2±0.2	0.6±0.2	1.9±0.5	1.3±0.1
Lysophosphatidylserine	0.9±0.4	0.8±0.2	1.0±0.3	1.4±0.4	1.2±0.3	1.4±0.2

Note. $p<0.05$: *compared to the control; $^{+}$compared to the control after 0.5 h; ^{x}compared to the control after 1 h.

dent activity of metabotropic glutamate receptors. However, phosphatidylinositol metabolites promote inhibition of neurotransmission. The release of hydrophobic diglycerides from phosphatidylinositol is of particular importance. They remain in the membrane and activate protein kinase C in the presence of phosphatidylserine. This enzyme prevents hyperactivation of Ca^{2+}-dependent processes [11]. Phosphatidylinositol is progressively accumulated in the frontal lobes of the cerebral hemispheres with an increase in the severity of HS. This process is related to desensitization and deactivation of receptors, whose agonists mediate phospholipase C expression. Previous studies revealed a delayed regional postischemic decrease in phospholipase C expression in the brain, which is associated with degradation of dendrites [13]. It cannot be excluded that disturbances in phosphatidylinositol metabolism during severe HS play a role in inactivation of protein kinase C. Moreover, the protective effect of protein kinase C is less pronounced at this stage of HS. These changes are related to depletion of phosphatidylserine, which serves as a protein kinase C cofactor in SM. Variations in the content of phosphatidylinositol and phosphatidylserine in SM from the frontal lobes of the cerebral hemispheres during severe HS are also of importance. Our conclusion is confirmed by published data that the loss of protein kinase C activity serves as prognostic criterion for lethal injury to neurons in cerebral ischemia [3].

Depletion of phosphatidylcholine is a major pathway for lipid bilayer destruction in SM from the frontal lobes of the cerebral hemispheres during HS. Phosphatidylcholine degradation in cholinergic neurons probably results from activation of phospholipase D with the formation of choline, which is involved in acetylcholine synthesis [2]. It was hypothesized that hyperactivation of cholinergic neurons is followed by a decrease in extracellular choline concentration, inhibition of choline release into adjacent cells, and impaired synthesis of membrane phosphatidylcholine [9]. Moreover, activation of phospholipase D is mediated by adrenergic stimulation [10] and glutamate in response to stimulation of metabotropic receptors [5]. The mechanisms of lipid bilayer destruction in SM from the frontal lobes of the cerebral hemispheres are probably observed at the initial stage of HS. Phosphatidylcholine degradation in SM during the delayed period of HS may be also associated with activation of phospholipase A_2 due to stimulation of NMDA receptors [9].

A specific feature of variations in the PL composition of SM from the frontal lobes of the cerebral hemispheres during HS is the increase in phosphatidylethanolamine content. The observed changes are accompanied by a decrease in phosphatidylcholine content at the early stage of HS. These data reflect impairment of phosphatidylethanolamine methylation, which contributes to PL conversion into phosphatidylcholine. Phosphatidylethanolamine accumulation in SM is probably related to the increase in intracellular Ca^{2+} concentration. Ca^{2+} may inactivate the enzyme regulating phosphatidylethanolamine metabolism (N-methyltransferase) [12]. The pathogenetic significance of phosphatidylethanolamine accumulation in SM from the frontal lobes of the cerebral hemispheres during HS arises from the fact that methylation is impaired in mental and neurological disorders. Moreover, a positive correlation was found between phosphatidylethanolamine N-methyltransferase activity and cognitive function [6].

The decrease in phosphatidylserine content in SM is an important pathogenetic factor for neurochemical disturbances in the frontal lobes of the cerebral hemispheres during HS. This PL serves as a cofactor of various enzymes and plays a role in cell excitability and transmembrane signaling [7]. A decrease in phosphatidylserine content in SM was accompanied by an increase in the ratio of phosphatidylcholine, which serves as the major substrate for its synthesis. These changes illustrate impairment of phosphatidylserine anabolism.

Our results show that HS is accompanied by variations in the PL composition of SM in the frontal lobes of the cerebral hemispheres. Hydrolysis of membrane phosphatidylinositol and its metabolites is a key pathogenetic stage of neurotransmitter-induced Ca^{2+}-mediated transmembrane signaling. Depletion of this PL in SM indicates that phosphatidylinositol derivatives play an important role in compensatory processes in the brain at the early stage of HS. Phosphatidylinositol accumulation in SM during severe HS is probably related to the instability of this neuroregulatory pathway. Depletion of membrane phosphatidylcholine initiates degenerative damage to neurons. Phosphatidylserine has a nootropic function. Therefore, the decrease in phosphatidylserine content during severe HS plays a major role in the progression of posthemorrhagic cognitive disorders. These changes, as well as the accumulation of membrane phosphatidylethanolamine, contribute to the development of encephalopathies. These data indicate that PL play the key role in the protective mechanisms of neurotransmission and development of dysregulation syndrome in the frontal lobes of the brain during HS.

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