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

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We studied phospholipid composition of brain synaptic membranes isolated from cats with severe hemorrhagic shock. Changes in the medulla oblongata were most pronounced and manifested in decreased content of phosphatidylcholine. Changes in the phospholipid composition of synaptic membranes in the frontal lobes included an increase in phosphatidylinositol content and reduced content of phosphatidylserine. Accumulation of phosphatidylethanolamine in synaptic membranes was found in both the medulla oblongata and frontal lobes. These data help to understand the mechanisms underlying exhaustion of compensatory reserves in brain cells during severe hemorrhagic shock.

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

Hemorrhagic shock (HS) with long-term and severe hypertension is accompanied by circulatory disorders, hypoperfusion, and hypoxia of the brain. The pathogenetic mechanisms of disturbances in the central nervous system (CNS) remain unclear. Phospholipids (PL) play an important role in physiological and biochemical processes in brain structures. Being the matrix for integral membrane proteins, they modulate activity of membrane-bound receptors and enzymes and regulate oxidative phosphorylation. PL determine the barrier function of membranes and maintain ion gradients. PL metabolism mediates the effect of transmitters, hormones, and other biologically active substances and is closely associated with intracellular signaling. These data suggest that modification of the lipid bilayer in nerve endings can seriously disturb synaptic processes during HS. Here we studied changes in the phospholipid composition of synaptic membranes (SM) in the medulla oblongata and frontal lobes isolated from cats with HS. These CNS structures are responsible for the regulation of breathing and circulation.

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MATERIALS AND METHODS

Experiments were performed on 18 cats weighing 3.0± 0.5 kg. The animals were anesthetized with nembutal (40 mg/kg intraperitoneally). HS was produced by the method of Wiggers and Fine. 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 The blood was withdrawn into a reservoir 30 min after heparin administration.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 by bloodletting and maintained at this level for 1 h. The control group included intact animals receiving heparin in a dose of 2000 U/kg. The medulla oblongata and frontal lobes were isolated 2 h after heparin administration (control cats) or 1.5 h after blood loss (experimental cats). SM were prepared as described elsewhere [12]. Total lipids were extracted by the method of Folch. PL were fractionated by thin-layer chromatography on Silufol UV-254 plates in a chloroform-methanol-7 N ammonia (12.4:4.6:1 v/v) system [5]. Chromatograms were densitometried on a Chromoscan-201 device (Joyce-Loebl).

Densitograms were analyzed on a Leitz-A.S.M. semiautomatic scanner. PL content was calculated in

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percents of the total peak area on densitograms. The results were analyzed by Student's *t* test.

RESULTS

In cats with HS the content of phosphatidylcholine in SM from the medulla oblongata decreased by 2.2 times compared to the control ($p<0.05$, Table 1). By contrast, the content of phosphatidylethanolamine increased by 23.8% ($p<0.05$).

The decrease in phosphatidylcholine content in SM of the medulla oblongata is probably related to activation of specific phospholipases. Since phosphatidylcholine inhibits lipid peroxidation [15], the decrease in its content can impair the antioxidant defense in synaptosomal membranes of the medulla oblongata during HS.

Phosphatidylethanolamine-specific methyltransferase plays an important role in the regulation of nerve cell activity. There are data on the existence of a phosphatidylethanolamine transformation pathway in membranes of cholinergic neurons. This pathway provides choline for acetylcholine synthesis and is regulated by methyltransferase [8]. Structural changes in SM of the medulla oblongata during HS make phosphatidylethanolamine less available for methylation. The increase in phosphatidylethanolamine content in SM of the medulla oblongata during HS probably reflects disturbances in metabolic transformations catalyzed by specific methyltransferase and reduced supply of SM with acetylcholine precursor choline.

Changes in SM of the frontal lobes during HS included an increase in the contents of phosphatidylethanolamine (by 18.5%, $p<0.01$) and phosphatidylinositol (by 1.9 times, $p<0.05$) and decrease in the concentration of phosphatidylserine (by 1.8 times, $p<0.01$, Table 1).

Our results indicate that phosphatidylserine content in the lipid bilayer of the frontal lobe SM decreases in cats with HS. It should be emphasized that phosphatidylserine activates protein kinase C [14], which acts as the key enzyme regulating cell activity. Exhaustion of this PL pool can impair synaptic transmission. Phosphatidylserine decarboxylase is involved in the formation of phosphatidylethanolamine and regulates conversion of this PL into phosphatidylcholine [14]. Decarboxylation of phosphatidylserine is activated under conditions of HS, which probably maintains a constant concentration of phosphatidylcholine in the frontal lobes and is responsible for accumulation of phosphatidylethanolamine. Our experiments showed that phosphatidylinositol is accumulated in SM of the frontal lobes during HS. Structural changes in these membranes produced by hypovolemia probably prevent the interaction of receptors with agonists regulating phosphatidylserine cleavage, which impairs transmembrane signaling.

Therefore, modification of the lipid bilayer in SM associated with the decrease in phosphatidycholine content was most pronounced in the medulla oblongata. It should be emphasized that the release of choline from phosphatidylcholine in cell membranes in CNS is important for acetylcholine synthesis under conditions of its intensive secretion and, hence, rapid utilization of its precursor choline [11]. At the same time, intensive utilization of choline-containing PL produces damages to cell membranes, impairs functional activity of cells, and causes their death. The formation of acetylcholine from phosphatidylcholine in cell membranes in CNS is regulated by phospholipase D activated via the stimulation of muscarinic receptors [11]. Previous studies showed that massive blood loss is accompanied by cholinergic hyperactivation [7]. Specific structural changes in SM of the medulla oblongata during HS related to the decrease in phosphatidylcholine content are determined by inten-

TABLE 1. Changes in PL Composition of Synaptic Membranes in Cats with HS (%, $M\pm m$, n=4-5)

PL fraction	Medulla oblongata		Frontal lobes	
	control	HS	control	HS
Phosphatidic acid	6.6±2.7	1.0±0.6	3.5±1.0	3.5±1.1
Phosphatidylethanolamine	30.3±3.6	39.4±1.0**	27.6±1.1	32.7±1.1*
Phosphatidylcholine	25.7±3.9	11.7±2.3**	31.8±4.9	25.2±2.2
Phosphatidylinositol	8.4±2.3	13.7±1.6	9.3±1.3	17.3±2.9**
Sphingomyelin	9.4±2.6	9.5±2.1	14.6±2.7	9.7±2.7
Lysophosphatidylethanolamine	3.7±0.5	4.3±0.9	2.8±0.7	2.7±0.3
Phosphatidylserine	4.1±0.6	3.6±1.4	5.0±0.8	2.7±0.6*
Lysophosphatidylserine	1.6±0.4	1.4±0.3	1.7±0.3	1.5±0.2
Lysophosphatidylcholine	2.1±0.6	3.9±0.8	1.9±0.5	1.3±0.2

Note. *$p<0.01$ and **$p<0.05$ compared to the control.

sive secretion of acetylcholine in cholinergic neurons localized in this brain region [2]. Our results indicate that the decrease in phosphatidylcholine content in SM during HS is the main factor producing damages to acetylcholine-synthesizing neurons constituting the bulbar parasympathetic nervous system.

During hypoxemia accompanying HS the release of norepinephrine and acetylcholine from nerve endings increases, while reactivity of the circulatory system to these neurotransmitters decreases due to occupation of receptors [7]. Our experiments revealed an increase in the content of phosphatidylinositol in SM, whose metabolism is activated by acetylcholine [13] and norepinephrine [1]. These data suggest that desensitization of cholino- and adrenoceptors of SM in the frontal lobes during HS is mediated by the same mechanism. Low concentrations of neuronal ATP involved in phosphatidylinositol phosphorylation can also contribute to accumulation of this PL [13]. The inhibition of phosphatidylinositol metabolism impairs transmembrane Ca^{2+} transport, which is regulated by phosphorylated metabolites of phosphatidylinositol [13]. Therefore, the decrease in phosphatidylinositol content in SM of the frontal lobes during can inactivate Ca^{2+} channels. These changes are associated with suppressed release of this neurotransmitter from nerve endings. Final stages of this process are regulated by Ca^{2+} [10].

Changes in the phospholipid composition of SM in the frontal lobes during HS include exhaustion of phosphatidylserine reserves. Being annular PL of opiate receptors phosphatidylserine binds opioid peptides. Phosphatidylserine decarboxylase inhibits binding of opiates to specific receptors on SM [3]. It can be hypothesized that reduced content of phosphatidylserine in SM of the frontal lobes in animals with HS inhibits binding of endogenous opiates with receptors. It is interesting that cholinergic activation accompanying massive blood loss [7] attenuates the influence of opiate peptides on brain cortical neurons due to the inhibition of their specific binding in cell membranes [6]. The decrease in phosphatidylserine content in SM of the frontal lobes during HS may be considered as the damaging factor that abolishes the effect of endogenous opiate peptides on neurotransmission in this brain region. Previous studies showed that the spinal cord is the main target for opiate peptides during hypotension accompanying massive blood loss [9]. Our results and published data indicate that partial opiate receptor agonists possessing activity of agonists and antagonists of opioid peptides hold much promise for the correction of HS.

The increase in phosphatidylethanolamine content in SM during HS was observed in various neurons of this brain region. It cannot be excluded that the intensity of phosphatidylethanolamine methylation in nerve endings also undergoes changes. Methylation of PL in CNS plays a particular role in transmission of nerve impulses mediated by amino acids [4]. It was hypothesized that the content of amino acids in neurons is regulated via methylation of PL. Intensive methylation of PL in SM suppresses the uptake of excitatory amino acids (EAA) in nerve endings. However, low-intensity methylation of PL abolishes the inhibition of EAA uptake. It should be emphasized that phosphatidylethanolamine metabolites that surround carrying molecules in the membrane determine normal functioning of the system responsible for high-affinity binding of transmitting amino acids [4]. Accumulation of phosphatidylethanolamine in brain SM during HS does not exclude the possibility that its methylation is decelerated and, therefore, neurotoxic activity of EAA increases.

Our results indicate that changes in the phospholipid composition of SM in the brain during severe HS are characterized by considerable regional differences, which is determined by structural and functional characteristics of neurons. These data suggest the existence of various mechanisms that underlie the formation of damages to CNS during disturbances in PL metabolism. Exhaustion of phosphatidylcholine reserves is one of the main damaging factors for neurons in the medulla oblongata. Therefore, hyperactivation of the cholinergic nervous system plays an important role in the pathogenesis of functional disorders in CNS. Modification of the lipid bilayer in SM of the frontal lobes determined by dysregulation of phosphatidylinositol and phosphatidylserine metabolism indicates that neurotransmission mediated via Ca^{2+} channels is vulnerable to damages. Under these conditions, the contribution of opiate peptides in the regulation of nerve-impulse transmission decreases. Accumulation of phosphatidylethanolamine in nerve endings of these brain regions can be associated with changes in the activity of amino acid-ergic receptors. These data elucidate the mechanisms underlying exhaustion of compensatory reserves in brain cells during severe HS.

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