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Changes in Phospholipid Composition of Cardiomyocyte Plasma Membranes during Hemorrhagic Shock

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Changes in the phospholipid composition of cardiomyocyte plasma membranes during hemorrhagic shock suggest that disturbances in phosphatidylethanolamine metabolism serve as one of the major factors for myocardial alteration in shock. Depletion of membrane phosphatidylcholine causes destruction of cardiomyocytes. The enhanced breakdown of membrane sphingomyelin at the late stage of hemorrhagic shock is considered as a mechanism, which induces apoptosis in cardiomyocytes and Ca^{2+} accumulation in these cells. A simultaneous increase in the content of membrane phosphatidylserine is the mechanism of activation of opioid receptors, which plays a compensatory role.

Key Words: *phospholipids; plasma membranes; cardiomyocytes*

Membrane phospholipids (PL) largely determine functional activity of tissues and organs by playing a role in cell excitation and communication. The metabolism and physical state of PL provide normal function of enzymatic and receptor systems in the plasmalemma and maintain the ion gradient. Ligand binding to cell surface receptors increases PL hydrolysis due to activation of various phospholipases. Receptor-mediated disintegration of small amounts of membrane PL mediates intracellular signal transduction by secondary messengers. PL metabolites regulate the intracellular calcium transport system and activate protein kinase C. They serve as secondary messengers of signaling, which induces differentiation of cells, suppression of growth, and apoptosis. Dysregulation of PL metabolism is followed by cell membrane injury and induces impairment of cell signaling, excitotoxicity of excitatory amino acids (EAA), and intensification of apoptosis.

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The composition of PL in cardiomyocyte plasmalemma was studied at various stages of hemorrhagic shock (HS) to evaluate the role of variations in membrane PL metabolism in the pathogenesis of myocardial injury.

MATERIALS AND METHODS

Experiments were performed on 25 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 The blood was withdrawn into a reservoir 30 min after heparin administration.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 and maintained at this level for 30 min and 1 h. The control group consisted of intact animals receiving heparin in the specified dose. Then these animals were euthanized with nembutal in a dose of 90 mg/kg and the heart was removed (1, 1.5, and 2 h after heparin administration in control animals and 0.5, 1, and 1.5 h after bleed-

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ing in the experimental cats). Cardiomyocyte plasma membranes were isolated as described elsewhere [9]. Total lipids were extracted by the method of Folch. PL were fractionated by thin-layer chromatography on Silufol UV-254 plates using a chloroform–methanol–7 N ammonia system (12.4:4.6:1.0 v/v). Chromatograms were densitometried on a Chromoscan-201 device (Joyce-Loebl) and analyzed on a semiautomatic image scanner (Leitz–A.S.M). The results were analyzed by Student's *t* test.

RESULTS

Changes in PL composition of cardiomyocyte plasma membranes were observed after 30-min blood loss: an increase in the content of phosphatidylethanolamine and decrease in the concentration of phosphatidylcholine (by 1.3 and 1.4 times, respectively (compared to the control); $p<0.01$, Table 1). After 1-h blood loss, the PL composition of cardiomyocyte plasma membranes was characterized by a decrease in the content of sphingomyelin (by 2.3 times compared to the control, $p<0.01$) and increase in relative phosphatidylserine content (by 1.5 times compared to the control, $p<0.05$). Variations in PL composition of cardiomyocyte plasma membranes 1.5 h after the start of bleeding were similar to those observed by the 30th minute. During this period, the content of phosphatidylethanolamine increased by 1.9 times ($p<0.01$), while the concentration of phosphatidylcholine decreased by 1.6 times compared to the control ($p<0.01$). It should be emphasized that PL composition of cardiomyocyte plasma membranes in control animals depended on the period of the study. In cats of the control group, phosphatidylethanolamine content 1.5 h after the start of bleeding was lower than 0.5 and 1 h after the start of blood loss (by 1.4 and 1.6 times, respectively; $p<0.02$-0.01). Phosphatidylcholine content in cats of the control group for 1-h blood loss was 1.2-fold lower than that in control animals for 30-min and 1.5-h blood loss ($p<0.05$-0.01). Sphingomyelin content in cats of the control group for 1-h and 1.5-h blood loss was higher than that in control animals for 30-min blood loss (by 2 times; $p<0.05$-0.02). These differences probably reflect substrate specificity of heparin in modeling of HS.

Our results indicate that metabolic disturbances of phosphatidylethanolamine and phosphatidylcholine in plasma membranes serve as a key mechanism for structural damage to cardiomyocytes at the early stage of HS. It is important to evaluate the influence of adrenergic stimulation on metabolism of these PL. The strength of adrenergic stimulation increases sharply at the early stage of HS. The role of β-adrenergic hyperstimulation of cardiomyocytes for the regulation of membrane phosphatidylethanolamine catabolism is determined by its capacity to increase Ca^{2+} entry into the cytosol [3]. These ions inhibit N-methyltransferase, the enzyme regulating the major catabolic pathway of intracellular phosphatidylethanolamine (methylation with the formation of phosphatidylcholine) [10]. The accumulation of phosphatidylethanolamine in cardiomyocyte plasma membranes is accompanied by a decrease in the content of phosphatidylcholine at the early stage of HS. These changes can be considered as the impairment of phosphatidylethanolamine methylation, which leads to important pathogenetic consequences. For example, phosphatidylethanolamine methylation is related to regulation of functional activity of glutamatergic neurons (abundant in the myocardium). Phosphatidylethanolamine metabolites determine activity of the EAA transport protein. High degree of phosphatidylethanolamine methylation is accompanied by the reduced transport of EAA across the membrane. By contrast, inhibition of EAA uptake by the cell is less pronounced at low degree of PL methylation [2]. Glutamate induces an increase in Ca^{2+} concentration in the cardiomyocyte sarcoplasm, which positively correlates with the increase in HR [15]. Metabolic changes in membrane phosphatidylethanolamine probably serve as the major cause of tachycardia, which is accompanied by a decrease in the volume of residual blood in the ventricles (as reported for the initial stage of HS).

Stimulation of α_1-adrenoceptors activates phospholipase D, a phosphodiesterase hydrolyzing phosphatidylcholine to phosphatidic acid and choline (acetylcholine precursor) [12]. Activation of phospholipase D is also observed during the interaction of acetylcholine with muscarinic receptors. Therefore, adrenergic and cholinergic hyperactivation of the myocardium at the early stage of HS serves as a mechanism of cardiomyocyte alteration related to the loss of phosphatidylcholine (major membrane PL), which results in the increase in membrane permeability. Cation efflux (primarily potassium) into the extracellular space is accompanied by disintegration of T-tubules into vacuoles, which impairs signal transduction from the sarcolemma to the contractile system of cardiomyocytes [6]. Hence, depletion of membrane phosphatidylcholine at the early stage of HS plays an important pathogenetic role and induces cardiomyocyte degeneration.

Phosphatidylcholine metabolites exhibit high biochemical activity. Phosphatidic acid released from phosphatidylcholine potentiates generation of superoxides, which increases activity of NADPH oxidase [11]. On the other hand, adrenergic stimulation led to enrichment of phosphatidylcholine with another metabolite, arachidonic acid [7]. This substance mediates hydrolysis of sphingomyelin due to activation

of neutral sphingomyelinase [8]. The increase in arachidonic acid concentration due to phosphatidylcholine breakdown, stimulation of intracellular oxidation, and subsequent activation of neutral sphingomyelinase [8] probably contribute to sphingomyelin depletion in cardiomyocyte plasma membranes after 1-h blood loss. Sphingomyelin hydrolysis is accompanied by the formation of ceramide, whose accumulation serves as a specific sign for increased apoptosis. Published data show that ceramide can stimulate intracellular Ca^{2+} influx through potential-dependent Ca^{2+} channels [14]. Hence, enhanced cleavage of sphingomyelin at the late stage of HS contributes to Ca^{2+} accumulation in cardiomyocytes.

Taking into account the fact that membrane phosphatidylserine can activate opioid receptors [1], accumulation of phosphatidylserine in the cardiomyocyte plasmalemma after 1-h blood loss probably increases the opioid activity, which has a modulatory effect on heart function. This fact is of considerable importance, because activation of opioid receptors mediates several reactions of the myocardium (*e.g.*, decrease in adrenergic influences on the heart and reduction of vagal bradycardia) [5]. The recovery of phosphatidylethanolamine and phosphatidylcholine in myocardial plasma membranes at this stage of HS probably results from the modulatory effect of opioids on adrenergic and vagal innervation of the heart. Phosphatidylserine accumulation of the plasmalemma plays an important role in induction and maintenance of compensatory processes, which contributes to recovery of the major structural components in the cardiomyocyte plasmalemma.

Ca^{2+} overload of cardiomyocytes is one of the key factors for heart injury during HS [13]. It can be expected that after 1.5-h blood loss, excessive intracellular Ca^{2+} (inhibiting methylation of phosphatidylethanolamine [10]) will result in an increase in the content of this PL in the cardiomyocyte plasmalemma and decrease in the concentration of membrane phosphatidylcholine. Activation of EAA is partly related to inhibition of phosphatidylethanolamine methylation [2]. Metabolic disturbances of the membrane PL probably play an important role in EAA toxicity in cardiomyocytes at the late stage of HS.

The type of changes in some classes of PL indicates that specific damage to the lipid bilayer of myocardial cell membranes depends on the stage of shock. Myocardial injury during shock is mainly associated with metabolic disturbances of membrane phosphatidylethanolamine, whose N-methylation serves a regulatory mechanism for intracellular calcium transport. The contractile apparatus of cardiomyocytes remains practically unchanged at the early stage of HS. Accumulation of membrane phosphatidylethanolamine serves as a risk factor for tachycardia. Cardiomyocyte degeneration is accompanied by disintegration of myofibrils at the late stage of hypovolemia [4]. These data suggest that reduced metabolism of membrane phosphatidylethanolamine at the late stage of HS contributes to Ca^{2+} overload of cells, which causes heart dysfunction. Phosphatidylcholine depletion in the plasmalemma forms the basis for cardiomyocyte destruction. The decrease in the content of membrane sphingomyelin plays a pathogenetic role in Ca^{2+} accu-

TABLE 1. PL Composition of Cardiomyocyte Plasmalemma in Cats (%, *M*±*m*)

Time, h	Group	Phosphatidic acid	Phosphatidylethanolamine	Phosphatidylcholine	Phosphatidylinositol	Sphingomyelin	Lysophosphatidylethanolamine	Phosphatidylserine	Lysophosphatidylcholine	Lysophosphatidylserine
0.5	Control (*n*=5)	3.1±1.0	28.4±1.2	40.9±1.7	10.9±0.9	4.5±1.3	3.0±1.6	4.2±1.4	2.2±0.6	3.0±1.1
	Shock (*n*=4)	3.6±1.6	37.6±1.8*	30.0±3.0*	12.6±1.1	6.4±1.1	1.6±0.3	5.8±1.4	1.7±0.3	1.4±0.3
1.0	Control (*n*=4)	2.0±0.8	32.8±2.0	33.7±1.0^{+}	10.2±0.5	8.8±0.6^{+}	3.2±0.4	4.6±0.7	2.3±0.5	2.1±0.5
	Shock (*n*=4)	1.6±0.4	33.2±4.0	35.6±4.1	10.9±1.1	3.8±0.7*	2.8±1.1	6.8±0.4*	2.8±1.3	2.2±0.4
1.5	Control (*n*=4)	3.3±0.2	20.2±2.3^{+x}	40.0±2.9^{x}	11.2±2.6	8.6±0.9^{+}	4.1±0.9	5.6±1.1	3.7±0.6	2.3±0.2
	Shock (*n*=4)	4.7±1.1	37.7±2.0*	24.4±4.1*	13.4±2.8	6.2±1.3	2.5±0.6	6.6±1.9	2.3±0.5	2.3±0.2

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

mulation in cardiomyocytes and activation of apoptosis. Taking into account the role of phosphatidylserine in opioid receptor activation, the increase in its content in cardiomyocyte plasma membranes probably potentiates the effect of opioids on the myocardium at the delayed stage of HS. The observed changes in PL metabolism in cardiomyocyte plasma membranes indicate that they plays the key role in the development of myocardial dysregulation during HS.

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