4. M. Sh. Avrushchenko, T. L. Marshak, V. I. Fateeva, *et al.*, *Anest. Reanimatol.*, No. 2, 43-46 (1993).
5. M. Sh. Avrushchenko, T. M. Solomatina, A. M. Gurvich, and I. V. Viktorov, *Byull. Eksp. Biol. Med.*, **114**, No. 8, 176-179 (1992).
6. A. M. Gurvich, E. A. Mutuskina, and Yu. V. Zarzhetskii, *Anest. Reanimatol.*, No. 5, 6-9 (1994).
7. V. G. Korpachev, S. T. Lysenkov, and L. Z. Tel', *Pat. Fiziol.*, No. 3, 78-80 (1982).
8. S. P. Lysenkov, V. G. Korpachev, and L. Z. Tel', in: *Emergencies: Clinical Features, Pathogenesis, and Management* [in Russian], Novosibirsk (1982), pp. 8-13.
9. N. I. Nezlina, E. A. Mutuskina, and S. V. Tolova, *Zh. Vyssh. Nervn. Deyat.*, **37**, No. 4, 741-743 (1987).
10. A. I. Roitbak, *Glia and Its Role in Neural Activity* [in Russian], St. Petersburg (1993).
11. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, *Arkh. Pat.*, **38**, No. 5, 48-54 (1976).
12. V. P. Tumanov, *Arkh. Pat.*, **45**, No. 1, 3-12 (1983).
13. H. Hara, T. Sukamoto, and K. Kogure, *Prog. Neurobiol.*, **40**, 645-670 (1993).
14. K. Kogure and J. Kawagoe, in: *Maturation Phenomenon in Cerebral Ischemia*, Berlin (1992), pp. 15-22.
15. L. Z. Pevzner, *Exp. Neurol.*, **65**, 237-241 (1979).

Changes in the Phospholipid Composition of Cat Hepatocyte Plasma Membrane in Hemorrhagic Shock

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Progressive elimination of phosphatidylcholine from the hepatocyte plasma membranes is observed during the development of hemorrhagic shock in cats. The phosphatidylinositol content decreases 30 min after the start of bleeding and then gradually increases. The phosphatidylethanolamine content is increased on the 30th and 60th min of bleeding, while the content of phosphatidylserine increases 1 h after the start of bleeding. At the peak of hemorrhagic shock the major shifts are observed in the phosphatidylcholine and phosphatidylinositol contents.

Key Words: *phospholipids; plasma membranes; hepatocytes; hemorrhagic shock*

Changes in the structure and function of cell membranes are one of the major determinants of the pathogenesis of shock at the cellular and subcellular levels [10]. Therefore, it is important to evaluate the role of structural modifications occurring in the plasma membrane phospholipids, since they strongly determine functional activity of the cell [1,5,6,8,9]. Bearing in mind that the liver is a target organ of hemorrhagic shock, we analyzed changes in the phospholipid composition of the hepatocyte plasma membrane in hemorrhagic shock.

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MATERIALS AND METHODS

Experiments were performed on cats (n=34, body weight 3.0±0.5 kg) under Nembutal anesthesia (40 mg/kg intraperitoneally). Hemorrhagic shock was produced by the method [16]. The animals were injected with 2000 units/kg heparin 30 min prior to bleeding. Blood pressure was reduced to 40 mm Hg within 30 min and maintained at this level for 1 h. Intact cats given the same dose of heparin served as the control. Liver was incised after perfusion with cold 1 mM $NaHCO_3$ 30 min, 1, and 1.5 h (experimental cats) and 1, 1.5, and 2 h (control cats) after the start of bleeding. Hepatocytes were isolated as described [4], and total lipid extraction was performed by the method [13]. Phospholipids were frac-

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tionated by thin-layer chromatography on Silufol UV-254 plates using a chloroform:methanol:acetic acid:water (25:15:4:2) mixture [15]. Chromatograms were processed in a Chromoscan-201 densitometer (Joyce-Loebl) and a Zeitz—A.S.M. semiautomatic image analyzer. Statistical analysis was performed using Student's t test.

RESULTS

Thirty minutes after the start of bleeding the content of phosphatidylethanolamine (PE) increased 3-fold ($p<0.001$), while the contents of phosphatidylinositol (PI) and phosphatidylcholine (PC) decreased, respectively, by 38.7% ($p<0.05$) and 10 times ($p<0.001$) (Fig. 1, *a*). On the 60th min of bleeding, PE content was 1.3-higher ($p<0.05$, Fig. 1, *b*) than in the control and 1.8-fold lower ($p<0.001$) than that on the 30th min of bleeding ($p<0.001$). It should be mentioned that the phosphatidylserine (PS) content increased 10-fold over the control level ($p<0.001$). The PI content was 3-fold higher ($p<0.001$), while PC content was 6-fold lower than in the control ($p<0.001$), being 1.7-fold higher ($p<0.001$) than with that on the 30th min of bleeding. On the 90th min of bleeding, the PI content increased 6-fold ($p<0.001$), while the PC content was 3.5-fold lower ($p<0.001$) compared with the control.

Thus, changes in the phospholipid composition of the hepatocyte plasma membrane occurring during of hemorrhagic shock reflect both degradation and restoration processes in hepatocytes. The major degenerative alterations are associated with increased elimination of PC. It should be noted that PC possesses an antioxidant activity [3], and its elimination from the hepatocyte plasma membrane stimulates lipid peroxidation [2]. At the initial stage of hemorrhagic shock, the PI content in the hepatocyte plasma membrane decreased. Both PI and PC of the plasma membrane are responsible for the realization of the effects of hormones involved in the adaptation mechanisms [8,9] via G-proteins and activation of A_2, C, and D phospholipases, leading to profound changes in cell metabolism. Specifically, the products of PI and PC hydrolysis participate in the regulation of protein kinase C, the major enzyme controlling cell functions [11]. Since PC catabolism is associated with regulatory mechanisms requiring prolonged activation of protein kinase C [12], hydrolysis of PC is more important for the regulation of protein kinase C activity than hydrolysis of phosphoinositides [7]. It was suggested that the transient increase in the content of the PI hydrolysis products is necessary for the initiation of PC hydrolysis [6]. It should be noted that protein kinase C participates in the PC metabolism via phosphorylation of phospholipase D or G-protein coupling phospholipase D to the receptor [6]. It was suggested that the receptor-mediated activation of phospholipase C is also controlled by protein kinase C [6].

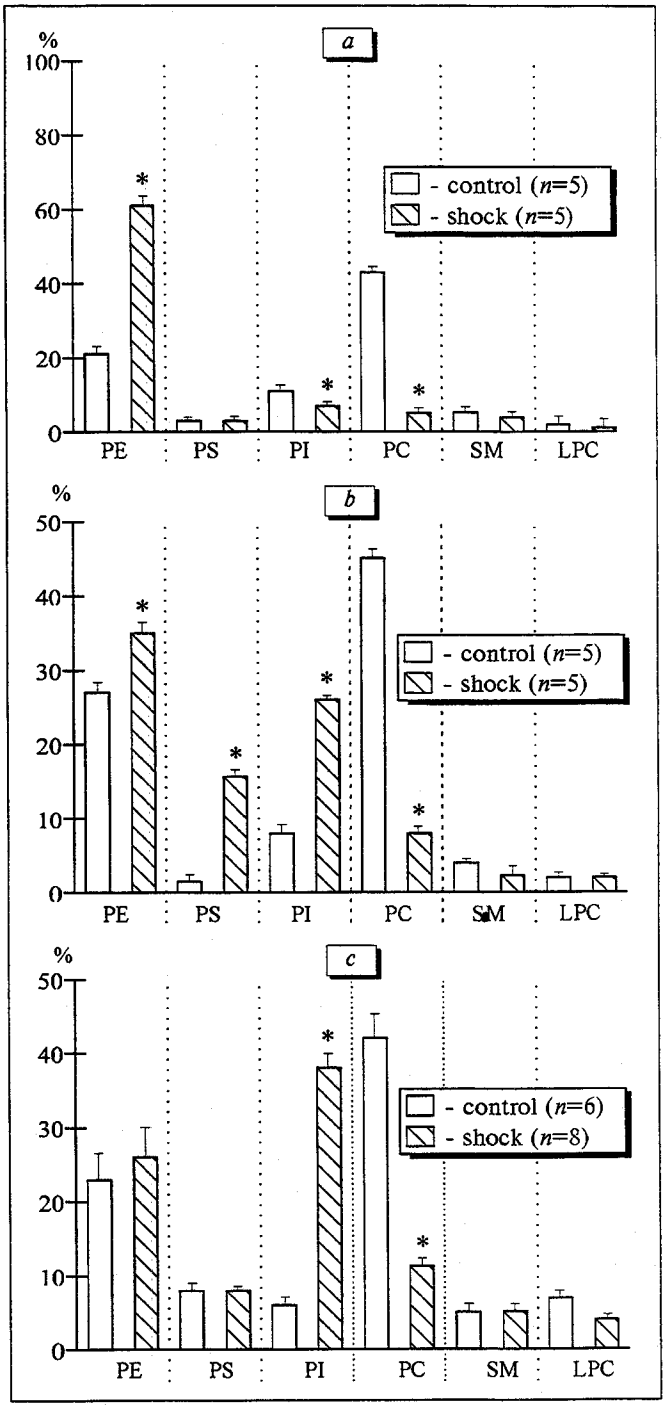

Fig. 1. Phospholipid composition of cat hepatocyte plasma membrane 0.5 h (*a*), 1 h (*b*), and 1.5 h (*c*) after the start of bleeding (*M±m*). *PE*) phosphatidylethanolamine; *PS*) phosphatidylserine; *PI*) phosphatidylinositol; *PC*) phosphatidylcholine; *SM*) sphingomyelin; *LPC*) lysophosphatidylcholine. *$p<0.05$ compared with the control; *n*) number of animals.

With this in mind, one can be expected that protein kinase C activity in hepatocytes is maintained by diglycerides formed as a result of PC hydrolysis, and protein kinase C may activate the catabolism of PC.

On the other hand, metabolism of PI is associated with calcium transmembrane transport, which is stimulated by enhanced PI metabolism [14]. Consequently, enhanced degradation of PI in the hepatocyte plasma membrane at the initial stage of hemorrhagic shock may stimulate calcium entry in hepatocytes.

The PE content of hepatocyte plasma membrane increased at the initial stage and to a greater extent at the late stage of hemorrhagic shock. Bearing in mind the finding that an increase in the plasma membrane PE content simulates calcium pump [17], it can be suggested that PE accumulation observed in our experiments represents a compensatory response aimed at removal of calcium from the cell. By the 60th min of bleeding, calcium outflow declined due to a decrease in the PE content. Increased plasma membrane contents of PE, PS, and PI may reflect enhanced regeneration aimed at maintaining the total mass of plasma membrane phospholipids. Presumably, PI accumulation in the hepatocyte plasma membrane at the terminal stage of hemorrhagic shock is associated with altered interaction between the agonists stimulating PI degradation and membrane receptors, which reduced the PI-mediated calcium entry. On the other hand, since PS is capable of binding to opioid peptides [1], it can be hypothesized that an increase in the PS content 1 h after the start of bleeding stimulates the binding of opioid peptides to hepatocytes.

Thus, modifications of the regulation of phospholipid turnover in the hepatocyte plasma membrane at the initial stage of hemorrhagic shock reflect enhanced compensatory responses to the damaging effect of an extreme stimulus. Then, regeneratory processes predominate, as evidenced by modifications of PI content and normalization of PE and PS contents. However, changes in the metabolism of PI and PC are not compensated even at the late stage of hemorrhagic shock.

REFERENCES

1. O. S. Belokoneva and S. V. Zaitsev, *Biokhimiya*, **58**, No. 11, 1685-1708 (1993).
2. E. B. Burlakova, A. V. Alekseenko, S. A. Aristarkhova, *et al.*, in: *Lipids of Biological Membranes* [in Russian], Tashkent (1982), pp. 16-23.
3. A. N. Zhuravlev, in: *Bioantioxydants* [in Russian], Moscow (1975), pp. 14-20.
4. A. V. Pospelova, in: *Modern Methods in Biochemistry* [in Russian], Moscow (1977), pp. 326-329.
5. G. S. Saatov, E. I. Isaev, M. Zh. Ergailova, *et al.*, *Role of Lipids in Metabolic Effects of Hormones* [in Russian], Tashkent (1990).
6. M. M. Billah and J. C. Anthes, *Biochem. J.*, **269**, 281-291 (1990).
7. J. H. Exton, *J. Biol. Chem.*, **265**, 1-4 (1990).
8. J. N. Fain, S.-H. Lin, J. Litsch, and M. Wallace, *Life Sci.*, **32**, 2055-2067 (1983).
9. N. R. Irving and J. H. Exton, *J. Biol. Chem.*, **262**, 3440-3443 (1987).
10. R. R. Kirby, *Int. Anesthesiol. Clin.*, **25**, 19-35 (1987).
11. Y. Nishisuka, *Science*, **233**, 305-312 (1986).
12. J. W. Putney, D. L. Aub, C. W. Taylor, and J. E. Merutt, *Fed. Proc.*, **45**, 2634-2638 (1986).
13. A. Rethy, V. Tomasi, and A. Trevisani, *Arch. Biochem.*, **147**, 36-40 (1971).
14. M. C. Sekar and L. M. Hokin, *Membr. Biol.*, **89**, 193-210 (1986).
15. V. P. Skipsky and V. Barklay, *Methods Enzymol.*, **14**, 530 (1969).
16. C. T. Wiggers, *Physiology of Shock*, London (1950).
17. P. L. Yeagle, *FASEB J.*, **3**, 1833-1842 (1989).