Dose-dependent Influence of Buprenorphine on the Phospholipid Composition of Cat Hepatocyte Plasma Membranes in Hemorrhagic Shock

G. F. Leskova and V. I. Udovichenko

UDC 616-001.5-001.36-092.9-085.31:
[547.95:547.943].015.21/.23

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 118, № 8, pp. 164-165, August, 1994
Original article submitted August 17, 1993

It is established that buprenorphine (0.3 mg/kg) induces considerable alterations in the phospholipid composition of hepatocyte plasma membranes as a result of phosphatidylserine accumulation and a considerable loss of sphingomyelin and lysophosphatidylserine. When administered in a dose of 0.03 mg/kg, buprenorphine facilitates normalization of the phosphoinositol turnover in hepatocyte plasma membranes.

Key Words: *buprenorphine; hemorrhagic shock; hepatocyte plasma membranes; phospholipids*

Endogenous opioid peptides are known to be a factor in the pathogenesis of shock. They cause marked venodilation, thus cutting off a considerable volume of blood from the central and peripheral circulation. It is known that antagonists of opiate receptors improve the function of the cardiovascular system during shock [6]. However, in some cases naloxone has no effect on this system and on the survival of animals [16]. On the other hand, by reducing opiate analgesia, naloxone can sometimes induce lethal changes in the function of the cardiovascular system [5]. Different results have been obtained in clinical studies of the consequences of naloxone administration during shock [3,12]. At the same time, it has been reported that in various models of shock agonists and antagonists of opioid peptides elicit a protective effect on hemodynamics [4]. Administration of these preparations during shock resulted in analgesia with improvement of cardiovascular function. Buprenorphine (BPN) is one of these preparations [4]. In this study we examined the effect of BPN on the phospholipid composition of hepatocyte plasma membranes in hemorrhagic shock. This is of interest because BPN is a highly lipophilic compound and rapidly dissolves in the cell membrane lipids [9], owing to which it can directly influence the lipid component of the plasma membrane.

Laboratory of Pathophysiology of Shock, Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow. (Presented by G. N. Kryzhanovskii, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were performed on 24 cats (3±0.5 kg) under ethanol-sodium anesthesia (40 mg/kg intraperitoneally). Hemorrhagic shock was performed by the method of Wiggers-Fine. Blood coagulation in the catheters was prevented by administration of 2000 U/kg heparin (HP). Thirty minutes after HP administration, blood was drained to attain a blood pressure of 40 mm Hg, and this pressure was maintained during a 1-h time period. The effect of BPN (0.03 and 0.3 mg/kg) was studied against the background of hemorrhagic shock. Intact animals treated with the same dose of BPN served as controls. The material for investigation was collected 2 h after anesthesia. After isolation of hepatocyte plasma membranes [1] and after extraction of total lipids [14], phospholipids were fractionated

0007-4888/94/0008-0856$12.50

by thin-layer chromatography on Silufol-254 plates in a chloroform:methanol:acetic acid:water system (25:15:4:2) [15]. Chromatograms were read in a Chromoscan-201 densitometer (Joyce-Loebl, England). The results were statistically analyzed using Student's t test.

RESULTS

In hemorrhagic shock, the major changes in the phospholipid composition of hepatocyte plasma membranes are associated with phosphatidylcholine and phosphatidylinositol (Table 1): the phosphatidylcholine content decreased 3.5-fold ($p<0.001$) and the phosphatidylinositol content increased 6.2-fold ($p<0.001$). When administered in hemorrhagic shock in a dose of 0.3 mg/kg, BPN induced considerable alterations in the phospholipid composition of hepatocyte plasma membranes: the phosphatidylserine content increased 4-fold ($p<0.001$), while the sphingomyelin and lysophosphatidylcholine contents decreased 6.7- ($p<0.01$) and 35.5-fold ($p<0.02$), respectively. On the other hand, when BPN was administered in a dose of 0.03 mg/kg, it had a beneficial effect on the phosphatidylinositol content of hepatocyte plasma membranes: a 30% normalization ($p<0.01$) was observed.

Thus, administration of BPN in a dose of 0.3 mg/kg against the background of hemorrhagic shock has a significant effect on the phospholipid composition of hepatocyte plasma membranes. In this connection, the increase in the phosphatidylserine content is of interest. It is known that the lipid phase is a matrix for the cell membrane receptors and is important in the agonist-receptor interaction. It was found that phosphatidylserine, an important component of opiate receptors [2], potentiates the binding of opioid peptides to receptors. Direct interaction of opioid peptides with phosphatidylserine has been demonstrated [17]. With this in mind, it can be speculated that the administration of BPN in a dose of 0.3 mg/kg facilitates the binding of opiates to hepatocyte plasma membrane receptors, notably due to the increase in the phosphatidylserine content.

On the other hand, in a dose of 0.3 mg/kg BPN induces a considerable loss of the sphingomyelin content of hepatocyte plasma membranes. There is evidence that sphingomyelin and its derivatives are involved in signal transduction [13]. It is hypothesized that sphingomyelin turnover is similar to that of phosphatidylinositol [8], but lasts for a longer time and may be implicated in more prolonged cellular alterations. Degradation of sphingomyelin leads to inactivation of protein kinase C [10]. On the other hand, it should be noted that the accumulation of sphingosine, a cytotoxic derivative of sphingomyelin, to concentrations inhibiting protein kinase C may result in cell necrosis [7].

Of interest is the effect BPN has on the lysophosphatidylcholine content. It is known that lysophosphatidylcholine modulates the activity of some membrane enzymes. Proceeding from the detergent properties of this compound, we assumed that its role in enzyme activation consists in solubilization of the membrane with subsequent stimulation of the enzyme-substrate reaction [11]. Thus, it is obvious that the drop in the lysophosphatidylcholine level of hepatocyte plasma membranes under the influence of BPN (0.3 mg/kg) may be reflected in the activity of the enzymes whose mechanisms of activation involve lysophosphatidylcholine.

Partial restoration of the phosphatidylinositol content of the plasma membrane was a specific feature of BPN administration in a dose of 0.03 mg/kg against the background of hemorrhagic shock. However, it is known that the metabolism of phosphatidylinositol is associated with transmembrane signal transduction and protein kinase C activation. Taking this into account, one can assume that partial correction of the phosphatidylinositol content in hepatocyte plasma membranes promotes normalization of cell metabolism when this dose of BPN is administered during the treatment of hemorrhagic shock.

TABLE 1. Phospholipid Composition (%) of Hepatocyte Plasma Membranes in the Cat ($M\pm m$)

Animals	PE	PS	PI	PC	SM	LPC	Other PL
Control (6)	23.9±5.8	7.9±2.0	6.0±1.1	42.3±4.2	4.7±1.5	7.1±3.4	8.0±1.7
Shock (8)	25.8±5.0	7.7±0.6	37.2±3.1*	12.1±3.3*	5.0±1.2	3.9±1.4	8.3±0.9
Shock + BPN 0.03 mg/kg (5)	37.1±5.8	6.6±1.7	23.2±2.8**	14.5±1.5*	4.1±1.1	4.4±1.5	10.2±2.0
Shock + BPN 0.3 mg/kg (5)	25.5±3.4	31.6±1.7**	30.8±2.3*	7.1±1.0*	0.7±0.2**	0.2±0.05**	4.0±0.5

Note. PE: phosphatidylethanolamine; PS: phosphatidylserine; PI: phosphatidylinositol; PC: phosphatidylcholine; SM: sphingomyelin; LPC: lysophosphatidylcholine; PL: phospholipids. The significance of differences in comparison with the control is indicated with one asterisk and with the parameters during shock with two asterisks. The number of animals is given in parentheses.

Thus, when administered in a dose of 0.3 mg/kg under conditions of hemorrhagic shock, BPN induces considerable alterations in the phospholipid composition of hepatocyte plasma membranes and can aggravate disorders of liver function. On the other hand, administration of BPN in a dose of 0.03 mg/kg has a protective effect on hepatocytes.

REFERENCES

1. A. V. Pospelova, *Modern Methods of Biochemistry* [in Russian], Moscow (1977), pp. 326-329.
2. L. G. Abood *et al., Biochim. Biophys. Acta,* **530**, 35-46 (1978).
3. F. Bonnet *et al., Crit. Care Med.,* **13**, 972-975 (1985).
4. M. D. J. Donaldson *et al., Circ. Shock,* **25**, 209-221 (1988).
5. F. Guarascio *et al., Brit. Med. J.,* **288**, 363-364 (1984).
6. N. Gurll, *Adv. Shock Res.,* **10**, 63-71 (1983).
7. Y. A. Hannun, *Science,* **235**, 7617-7623 (1987).
8. Y. A. Hannun and R. Bell, *Ibid.,* **243**, 500-507 (1989).
9. M. Kajiwara *et al., Japan J. Pharmacol.,* **40**, 96-101 (1986).
10. R. N. Kolesnik, *J. Biol. Chem.,* **264**, 7617-7623 (1989).
11. S. Mookerjeo and J. W. M. Yung, *Can. J. Biochem.,* **52**, 1053-1066 (1974).
12. W. P. Peters, *Lancet,* **1**, 529-532 (1991).
13. T. Okazaki, R. M. Bell, and Y. A. Hannun, *J. Biol. Chem.,* **264**, 19076-19080 (1989).
14. A. Rethy *et al., Arch. Biochem.,* **147**, 36-40 (1971).
15. V. P. Skipsky and M. Barclay, *Meth. Enzymol.,* **14**, 530-598 (1969).
16. K. Van Der Meek *et al., Europ. J. Pharm.,* **124**, 299-308 (1986).
17. F. Vita *et al., Int. J. Peptide Protein Res.,* **28**, 220-229 (1986).