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The Effect of Hemorrhagic Shock on the Phospholipid Composition of Blood Plasma of Anesthetized Dogs[1] (37747)

HENRY P. SCHWARZ, LORRAINE DREISBACH, AND JOHN J. SPITZER

Department of Clinical Pathology, Philadelphia General Hospital, Philadelphia, Pennsylvania 19104; and Department of Physiology and Biophysics, Hahnemann Medical College, Philadelphia, Pennsylvania 19102

Recent studies on the effect of various types of physical or psychic stress on the phospholipid composition of blood plasma and tissues in humans and animals showed a significant increase of one particular phospholipid, phosphatidylglycerol (GPG), while other phosphatides were affected to a lesser extent or not at all (1). Some of these studies suggested a possible role of the brain in the regulation of phospholipids in stress. Assuming that adaptation to environmental and pathological stress may involve biological energy control and utilization, it was thought that the regulatory role of the brain in phospholipid metabolism in stress may be stimulated by changes of oxygen utilization of the brain. This problem was explored by examination of the plasma phospholipids in the carotid artery and internal jugular vein of patients with cerebrovascular diseases and various degrees of decreased cerebral blood flow and diminished cerebral oxygen consumption. This investigation showed that the GPG content of the plasma from the internal jugular vein was significantly greater than the GPG value of the plasma from the internal carotid artery. However, while there was some correlation between the A–V difference of GPG across the brain and decreased cerebral blood flow, or cerebral oxygen consumption no more definite relation between these parameters could be established (2). It was thought that more evidence for a possible role of the brain in the described changes of phospholipids in stress may be obtained from animal experiments allowing simultaneous examination of phosphatides across the brain and the splanchnic area of the circulation. It was decided to examine the phospholipid composition of the blood plasma from the femoral artery, the sagittal sinus and the hepatic vein before and after hemorrhagic shock.

Materials and Methods. The experiments were performed on 7 mongrel dogs. The animals were anesthetized by intravenous administration of sodium pentobarbital (35 mg/kg). A continuous infusion of the anesthetic was also maintained throughout the experiment in order to produce a relatively steady level of anesthesia. An arterial cannula was placed in the carotid artery to monitor blood pressure, another was introduced into the femoral artery for sampling of arterial blood and hemorrhaging the animal. Hepatic venous blood samples were obtained through a catheter introduced from the jugular vein and directed into the left hepatic lobe by manual controls through laparotomy. Sagittal sinus blood was taken through a catheter. One hour after the placement of all the catheters control blood samples were taken simultaneously from the femoral artery, hepatic vein and sagittal sinus vein. This was followed by a massive hemorrhage which consisted of removing approximately half of the circulating blood volume. Arterial blood pressure was monitored throughout the hemorrhage, and withdrawal

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of the blood was adjusted so that the post hemorrhagic mean arterial blood pressure equaled about 50% of the control value. Approximately 30 min following hemorrhage a second experimental set of blood samples was taken from the same sites. At the end of the experiment the location of all catheters was confirmed.

All blood samples were collected in heparinized tubes and kept and centrifuged in the cold. No heparin was administered into the animals.

Analysis of the phospholipid composition of the blood plasma from the femoral artery, sagittal sinus, and hepatic vein. The blood samples were centrifuged immediately and the plasma was frozen and stored, or when possible, extracted immediately with 20 vol of chloroform–methanol (2:1 v/v) for 24 hr at room temperature. No significant changes in the analyzed phospholipids were observed during the freezing and storage. The protein precipitate was filtered off on a sintered glass funnel and the solution was evaporated to dryness in nitrogen stream. The residue was dissolved in chloroform–methanol–water (60:30:45). This solution was passed through a glass column of 6 mm diameter packed with 2 g of Sephadex G-25 for removal of non-lipid impurities (3). The eluate was again evaporated to dryness in a stream of nitrogen. The residue was dissolved in 25 ml of chloroform. The separation of the phospholipid fractions and phospholipid analyses were carried out in duplicate as described elsewhere (4, 5). The purified lipid extract was passed through a column of 6 g silicic acid for removal of the neutral lipids and aliquots of the phospholipids thus obtained were subjected to mild alkaline hydrolysis with 0.03 *N* NaOH in ethanol for 20 min at 37°. The hydrolyzate was neutralized with ethyl formate and again evaporated in a nitrogen stream. The residue was distributed between 2 vol of isobutanol–chloroform (1:2, v/v) and 1 vol of water and the two phases were separated by centrifugation. An aliquot of the water phase containing the deacylated (alkali labile) phospholipids was placed on Whatman 3 MM paper and the glycerol phosphate components were separated first by descending chromatography in phenol saturated with water–acetic acid–ethanol for 16 hr. The solvents then were removed and separation in the second dimension was obtained by high voltage ionophoresis in pyridine–acetic acid–water at pH 3.6 using a current of 100–125 mA at 2000 V for 1.5 hr with a Savant apparatus. The chromatograms were dried and then sprayed with Ninhydrin reagent to locate amino lipids and afterwards with acid molybdate (4) to determine the phosphorus compounds. Choline containing compounds were detected with Dragendorff's reagents when necessary. Analyses of the alkali stabile fractions (plasmalogens, sphingomyelin, alkyl ethers) contained in the chloroform fraction were carried out as described by Dawson (4). The individual chromatographic spots were identified by R_f values found with deacylated authentic standards. In the case of GPG pure phosphatidylglycerol, which was isolated from human blood plasma (6), ^{14}C labeled GPG isolated from Scenedesmus (7), and chemically synthesized GPG (8) were used. Quantitation of the individual phospholipids was performed by digestion of the stained spots with 72% of perchloric acid and subsequent determination of inorganic phosphorus (9, 10).

Results and Discussion. Table I demonstrates the detailed composition of plasma phospholipids from the artery and veins before and after hemorrhagic shock. It is demonstrated that prior to the shock the concentrations of GPG, phosphatidyl ethanolamine, and phosphoinositides were significantly higher in the plasma from the sagittal sinus than in the plasma from the artery. The elevation of phosphoinositides may not be meaningful, because of limitation of the method (4). No similar increase of GPG or any other phospholipid was found across the splanchnic area (in the hepatic vein) under control conditions.

Effect of severe hemorrhage on phospholipids in arterial and venous plasma. Table I also shows that following the hemorrhage there was a small decrease of total lipid phosphorus and lecithin concentration in the arterial plasma. At the same time the arterial

TABLE I. Effect of Hemorrhage on Phospholipid Composition of Plasma in Various Parts of the Circulation of Anesthetized Dogs.[a]

	Arterial		Hepatic		Sagittal sinus	
Hemorrhage:	Before	After	Before	After	Before	After
No. of expts	7		7		7	
Total lipid phosphorus	2264 ± 65	2083 ± 79	2427 ± 139	2205 ± 123	2407 ± 93	2141 ± 104
Lecithin	1796 ± 65	1608 ± 91	1917 ± 87	1716 ± 98	1817 ± 79	1589 ± 89
Phosphatidyl ethanolamine	26 ± 2	24 ± 2	30 ± 3	28 ± 2	37 ± 3[cd]	34 ± 3[ce]
Phosphatidylserine	15 ± 1	14 ± 2	15 ± 2	12 ± 2	13 ± 1	13 ± 1
Cardiolipin	24 ± 2	24 ± 1	29 ± 4	27 ± 3	27 ± 1	24 ± 1
Phosphatidic acid	9 ± 1	12 ± 1	11 ± 2	12 ± 1	14 ± 2	15 ± 2
Phosphatidylglycerol	28 ± 2	38 ± 2[bd]	30 ± 2	41 ± 2	35 ± 2[bd]	52 ± 4[ce]
Phosphatidylinositide	61 ± 6	67 ± 4	80 ± 6	75 ± 7	94 ± 7[cd]	85 ± 6[be]
Inorganic phosphorus	11 ± 1	12 ± 1	13 ± 2	12 ± 2	13 ± 2	13 ± 1
X-2	19 ± 5	14 ± 1	17 ± 4	17 ± 3	14 ± 2	14 ± 4
Alkali labile unknowns	9 ± 2	11 ± 2	10 ± 2	12 ± 4	11 ± 2	11 ± 2
Choline plasmalogen	23 ± 3	25 ± 2	30 ± 4	27 ± 4	33 ± 4	34 ± 3
Ethanolamine plasmalogen	12 ± 2	14 ± 1	16 ± 2	15 ± 2	15 ± 2	16 ± 1
Serine plasmalogen	8 ± 1	7 ± 1	10 ± 1	9 ± 1	8 ± 1	9 ± 1
Unknown plasmalogen	3 ± 1	4 ± 1	6 ± 1	5 ± 1	4 ± 1	5 ± 1
Sphingomyelin	149 ± 16	146 ± 13	160 ± 19	141 ± 13	193 ± 14	172 ± 2
Acid labile unknowns	11 ± 1	12 ± 1	12 ± 2	9 ± 1	14 ± 3	13 ± 1
Alkyl ethers	31 ± 1	31 ± 1	31 ± 2	29 ± 2	38 ± 2	32 ± 3

[a] Values are expressed as micromoles per liter, mean ± SEM.
[b] $P < 0.05$.
[c] $P < 0.02$.
[d] Comparison with arterial values before hemorrhage.
[e] Comparison with arterial values after hemorrhage.

TABLE II. Differences in Plasma Phospholipid Concentration (μmoles/liter) Across the Brain.[a]

	Sagittal sinus minus arterial	
	Before hemorrhage	After hemorrhage
Total lipid phosphorus	143 ± 78	58 ± 46
Phosphatidyl ethanolamine	12 ± 4	10 ± 3
Phosphatidylglycerol	7 ± 2	12 ± 4

[a] Values are expressed as micromoles per liter, mean ± SEM; differences represent mean change ± SEM.

concentration of GPG increased significantly.

No significant differences were observed in any of the phospholipids across the splanchnic area following hemorrhage. The differences in plasma phospholipid concentrations across the brain before and after hemorrhagic shock are reviewed briefly in Table II.

It can be seen from Table II that while there was no significant difference of total lipid phosphorus across the brain following hemorrhage, the negative A–V difference (*i.e.*, release) for phosphatidyl ethanolamine still persisted and the difference for GPG even became markedly greater (from 7 ± 2 to 12 ± 4). This change of the difference of GPG across the brain after the hemorrhage is not quite significant statistically, because a few rather high differences of GPG across the brain existed already before the hemorrhagic shock.

It is not quite clear at this time what the physiological significance of the phospholipid release from the brain is under control conditions. Although decrease of total phospholipid concentration following hemorrhage has been observed previously (11) its importance is still obscure. It is of interest to note that GPG, which was shown to be associated with other forms of shock is elevated following hemorrhage and its source (at least in part) is the brain.

The biological significance of the increase of GPG in plasma after hemorrhagic shock can only be surmised at present. It is of interest to note in this connection that the increase of GPG in plasma after another stress (acceleration of rats) failed to occur in hypophysectomized animals and that a considerable elevation of GPG in the brain appeared instead (1). Recent *in vitro* studies by Eichberg *et al.* (12) showed that catecholamines such as L-norepinephrine increased the ^{32}P incorporation into GPG 4- to 6-fold in pineal culture systems. It thus may be hypothesized that the considerable increase of catecholamines found after hemorrhagic shock (11) may trigger an increase of GPG in the brain, which then may be released into the circulation. Discussing a probable function of GPG in stress it is well to emphasize that Macfarlene (13) and others (14) have given convincing evidence that a number of bacteria produce ortho-amino acid esters of GPG and phosphatidopeptides have been reported isolated from liver and other animal tissues (15). One may be tempted to postulate a possible release of polypeptide esters of GPG from the pituitary to be involved in adaptation to stress, but such an assumption may only be realized after extensive additional investigations.

Summary. The phospholipid composition of arterial, sagittal sinus and hepatic venous blood plasma was investigated in dogs under pentobarbital anesthesia both before and shortly after a severe hemorrhage. The total lipid P concentration in the artery decreased following hemorrhage, but the phosphatidylglycerol concentration increased. Only small and variable differences were observed across the splanchnic region either before or after the bleeding. A significant release of total lipid P, phosphatidyl ethanolamine, choline plasmalogen and phosphatidylglycerol was noted across the brain under control conditions. The individual phospholipids were continued to be released even following hemorrhage. The physiological significance of these changes will have to await further study.

1. Polis, B. D., Polis, E., DeCani, J., Schwarz, H. P., and Dreisbach, L., Biochem. Med. **2**, 286 (1969).

2. Schwarz, H. P., McHenry, L. C., Jr., Dreisbach, L., and Jafee, M. E., Proc. Soc. Exp. Biol. Med. **135**, 55 (1970).

3. Wells, M. A., and Dittmer, J. C., Biochemistry **2**, 1259 (1963).

4. Dawson, R. M. C., Biochem. J. **75**, 45 (1960).

5. Schwarz, H. P., Dreisbach, L., Polis, E., Polis,

B. D., and Soffer, E., Arch. Biochem. Biophys. **111**, 422 (1965).

6. Bishara, E., and Schwarz, H. P., Bacteriol. Proc. 23 (1969).

7. Schwarz, H. P., and Dreisbach, L., Biochim. Biophys. Acta **210**, 436 (1970).

8. Saunders, R. M., and Schwarz, H. P., J. Amer. Chem. Soc. **88**, 3844 (1966).

9. Sperry, W., Ind. Eng. Chem. (Anal. Ed.) **14**, 88 (1942).

10. Bartlett, C. R., J. Biolog. Chem. **234**, 466 (1959).

11. Spitzer, J. A., and Spitzer, J. J., J. Trauma **12**, 898 (1972).

12. Eichberg, J., Schwarz, M., Shein, H., and Hauser, G., Fed. Proc., Fed. Amer. Soc. Exp. Biol. (Abst.) 1337 (1972).

13. Macfarlane, M. G., *in* "Advances in Lipid Research" (R. Paoletti and D. Kritchevsky, eds.), Vol. 2, p. 91. Academic Press, New York (1964).

14. Houtsmuller, U. M. T., and Van Deenen, L. M. M., Biochim. Biophys. Acta **70**, 211 (1963).

15. Huggins, C. G., and Cohn, D. V., J. Biol. Chem. **234**, 257 (1959).

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