Phospholipid Metabolism In Patients with Cerebrovascular Disease[1] (34986)

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Recent studies of the effect of various types of stress on the phospholipid composition of blood plasma and tissues showed that whole body X-ray irradiation, or acceleration of rats up to 20*g* was regularly accompanied by a significant increase of one particular plasma phospholipid, namely, phosphatidylglycerol (GPG) (1). Humans subjected to such stress as sleeplessness, combat, or schizophrenia showed similar elevation of GPG in blood plasma. Other plasma phospholipids or total lipid phosphorus were affected by the stress to a lesser extent or not at all. Animal experiments aimed at elucidating the mechanism of those changes showed that the significant increase of plasma GPG found after the acceleration of normal rats failed to occur in hypophysectomized rats, but that instead a considerable elevation of GPG appeared in the brains of those animals after the acceleration. Since these experiments suggested a role of the brain in regulation of the phospholipid metabolism in stress, it was decided to examine the phospholipid composition of the blood plasma from the internal carotid artery and internal jugular vein in patients with cerebrovascular disease and to correlate the results with cerebral blood flow and cerebral oxygen consumption.

Material and Methods of Procedures. Patients with a history and symptoms of cerebrovascular disease were admitted to the Neurology Service of the hospital and studies were carried out by the Stroke Research Center. After receiving complete clinical and laboratory examinations, the patients who did not represent an undue risk and gave informed consent were subjected to the experimental procedures.

Experimental procedures. Analysis of the phospholipid composition of the blood plasma from the internal carotid artery and internal jugular vein. The blood specimens were obtained in conjuction with studies of the regional cerebral blood flow and cerebral oxygen consumption. The blood samples were centrifuged immediately and the plasma frozen and stored or, when possible, extracted immediately with 20 vol of chloroform–methanol (2:1 v/v) for 24 hr at room temperature. The protein precipitate was filtered off on a sintered-glass funnel and the solution was evaporated to dryness with a nitrogen stream. The residue was dissolved in chloroform–methanol–water (60:30:4.5). This solution was passed through a glass column of 6 mm diameter packed with 2 g of Sephadex G-25 for removal of nonlipid impurities (2). The eluate was again evaporated to dryness in a nitrogen stream. The residue was dissolved in 25 ml of chloroform. The analyses of phospholipids were carried out in duplicate with procedures described elsewhere (3, 4) so that details of the method may be omitted here. The purified total lipid extract was passed through a column of 6 g of silicic acid for removal of neutral lipids. Aliquots of the phospholipids thus obtained containing 100–500 μg of phosphorus 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

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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 deacylated (alkali-labile) phospholipids contained in the water phase was spotted on Whatman 3MM paper and the glycerol phosphate components were separated first by descending chromatography in phenol saturated with water–acetic acid–ethanol (50:5:6, v/v/v) for 16 hr. The solvents then were removed and resolution in the second dimension was accomplished by high-voltage ionophoresis in a buffer solution of pyridine–acetic acid–water (1:10:89) at pH 3.6 using a current of 100–125 mA at 2000 V for 1½ hr. The chromatograms were sprayed with ninhydrin to locate the amino lipids and afterwards with acid molybdate (5) to determine phosphorus compounds. Choline containing components were recognized by Dragendorff's reagents when necessary. Analysis of the alkali-stable lipids (plasmalogens, sphingomyelins, and alkyl ethers) was carried out by the original procedure of Dawson (3). The individual chromatographic spots were identified from the two-dimensional chromatograms of authentic standards. In the case of GPG a pure ^{14}C-labeled GPG isolated from Scenedesmus (6), pure phosphatidylglycerol isolated from pooled human plasma, and chemically synthesized GPG (7) were used as standards. Quantitation of the individual phospholipids was accomplished by digestion of the stained spot with 72% perchloric acid and subsequent determination of the inorganic phosphorus (8, 9).

Measurement of regional cerebral blood flow. The measurement of regional cerebral blood flow (rCBF) was based on the isotope-clearance technique of Lassen and Ingvar (10). The method used in our studies has been described by McHenry *et al.* elsewhere (11). The procedure was carried out in conjuction with cerebral angiography in the following manner: After the common carotid artery was punctured and under fluoroscopic guidance, a small catheter was passed into the internal carotid artery. Approximately 2 mCi of xenon-133 in 4 ml of saline was abruptly injected. The clearance of the isotope was monitored by eight scintillation detectors with 0.5 by 0.5 NaI crystals contained in probes in a collimator block at the side of the patient's head. Each of the eight detectors worked simultaneously and monitored the clearance of the isotope from a cylindrical area of the brain of 1.5 in. diameter. The signals from the detectors were recorded separately on multiple-channel magnetic tape. The clearance curves thus obtained were reproduced individually through a digital scaler and plotted on a chart recorder. The data taken from the curves were transferred to punch cards and analyzed on the IBM 360 Computer using a translation of the program developed by Sveinsdottir (12). In these studies the mean stoichastic regional

TABLE I. Phospholipid Composition of Blood Plasma in Cerebrovascular Diseases (mean ± SE).

	Arterial	Venous
Number of cases	35	35
Total μmoles lipid phosphorus/ liter plasma	1913 ± 56	2219 ± 64[a]
Percent distribution		
Lecithin	64.90 ± 0.61	65.46 ± 0.47
Phosphatidylethanolamine	2.11 ± 0.11	2.07 ± 0.08
Phosphatidylserine	0.86 ± 0.03	0.88 ± 0.04
Cardiolipin	1.41 ± 0.06	1.40 ± 0.06
Phosphatidic acid	0.64 ± 0.03	0.77 ± 0.04[a]
Phosphatidylglycerol	1.36 ± 0.04	2.07 ± 0.10[a]
Phosphatidylinositide	3.99 ± 0.16	4.13 ± 0.13
Inorganic phosphorus	0.62 ± 0.04	0.61 ± 0.03
Alkali-labile unknowns	1.31 ± 0.06	1.29 ± 0.07
Choline plasmalogen	2.19 ± 0.10	2.03 ± 0.08
Other plasmalogens	1.48 ± 0.07	1.39 ± 0.06
Sphigomyelin	14.85 ± 0.34	14.77 ± 0.30
Acid-labile unknowns	0.45 ± 0.04	0.38 ± 0.03
Alkyl ethers	1.55 ± 0.06	1.63 ± 0.06

[a] Significantly different from arterial value ($p < .01$).

cerebral blood flow value in cc/100 g/min are reported. These data are based on computations of Zierler (13). Comparison of the mean rCBF values with those derived from studies of cerebral blood flow by a modification of the Kety–Schmidt technique (14) showed that the data were comparable in patients with normal cerebral angiograms or with diffuse cerebrovascular disease.

Determination of cerebral oxygen consumption. Cerebral oxygen consumption was determined from the arterial and jugular venous oxygen difference times the mean regional cerebral blood flow. The blood specimens were obtained together when the samples for determination of phospholipids were drawn. This was done during the regional cerebral blood flow measurement. The oxygen determinations were carried out by the manometric method of Van Slyke.

Results and Discussion. Composition of the plasma phospholipids (see Table I). Analyses of the plasma phospholipids of 35 patients with cerebrovascular disease showed that the mean percentage of GPG of the plasma from the internal jugular vein, *e.g.*, 2.07% was 52% greater than that from the internal carotid artery, *e.g.*, 1.36%. Of the other phospholipids only phosphatidic acid showed moderate elevation of the mean per cent value in the venous blood while the remaining individual phospholipids showed quite similar figures in the plasma from both internal carotid artery and internal jugular vein. The total lipid phosphorus content of the venous plasma exceeded slightly that from the internal carotid artery. The considerable elevation of the mean GPG percentage in the venous plasma goes far beyond any possible error as deviation from the mean of duplicate determinations of GPG was found to be less than 5%. The significance of this finding is enhanced further if we consider that analyses (not given in Table I) of plasma samples from the brachial vein, which were obtained simultaneously with those from the jugular vein, did not show any differences from the values listed for arterial blood.

Regional cerebral blood flow and cerebral oxygen consumption (see Table II). The mean rCBF value in our 35 patients with cerebrovascular disease was 36.5 cc/100 g brain/minute. The mean rCBF value for normal individuals was 54.4 cc/100 g brain/minute (11). A single healthy volunteer examined in our series of experiments even showed a rCBF value of 67 cc/100 g brain/minute. The considerable decrease of regional cerebral blood flow found in these patients was accompanied by definite lowering of the cerebral oxygen consumption to a mean value of 2.2 cc O_2/100 g brain/minute as compared with normal figures of 3.5 cc O_2/100 g brain/minute observed by Kety and Schmidt (15) and many others (16).

Relation of the described changes of plasma phosphatidylglycerol to decreased oxygen consumption (see Fig 1). The correlation of the described changes of plasma phosphatidylglycerol to decreased oxygen consumption of the brain is illustrated in the scatter diagram (Fig. 1) showing the relation of the A–V difference of GPG to cerebral oxygen consumption in the individual cases. This diagram suggests that the decreased oxygen consumption is accompanied in some patients by relative increases of the A–V

TABLE II. Arterial–Venous Differences of Plasma Phosphatidylglycerol, Blood Flow (Stochastic Mean of Regional rCBF), and Oxygen Consumption in Cerebrovascular Diseases.

	Cerebrovascular diseases ($N = 35$)	Normal volunteer ($N = 1$)
Phosphatidylglycerol (moles/liter plasma)		
Arterial	25.8 ± 1.1	23.5
Venous	45.8 ± 2.5	33.4
Blood flow	36.5 ± 1.8	67
Oxygen consumption/100 g brain/min	2.16 ± 0.14	4.02
Vol. % arterial oxygen–venous oxygen	6.15 ± 0.39	6.0

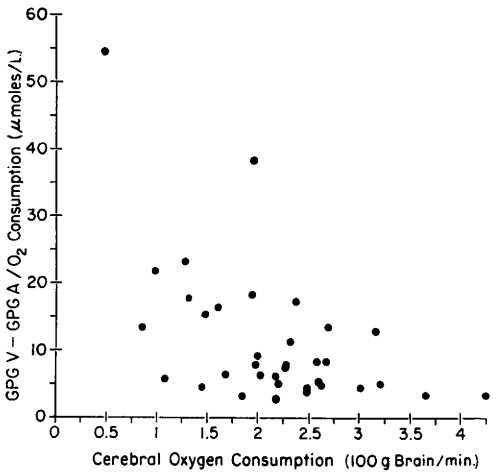

FIG. 1. Increase of phosphatidylglycerol (GPG) of internal jugular vein (V) over the internal carotid artery (A) to the cerebral oxygen consumption in patients with disturbed circulation of the brain.

difference of GPG. Statistical evaluation of the plotted data gives an r value of -0.387 indicating only a low correlation between these two parameters. It must be concluded, therefore, that other factors in addition to low oxygen consumption contribute to the plasma phospholipid changes in these patients with various types of impaired cerebral circulation.

Summary. The GPG content of the plasma from the internal jugular vein of patients with cerebrovascular disease was significantly greater than the GPG value of the plasma from the internal carotid artery. There was some correlation between the A–V difference of GPG and lowered cerebral oxygen consumption ($r = -0.387$), but no more definite relation between these two parameters could be established.

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