EFFECT OF CEREBRAL ISCHEMIA ON METABOLISM OF INDIVIDUAL PHOSPHOLIPID FRACTIONS

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A decrease in the intensity of metabolism both of total phospholipids (PL) and of their individual fractions was observed in the cerebral hemispheres 90 min after ligation of the common carotid arteries in rats, and the decrease was approximately equal in degree. In the diencephalon depression of total PL metabolism was due to depression of metabolism of the phosphatidylcholine and phosphoinositide fractions.

KEY WORDS: brain; ischemia; phospholipids.

The central nervous system (CNS), the chemical composition of which is distinguished by a higher concentration of phospholipids (PL) than in other tissues, is very sensitive to interruption of the cerebral circulation [5, 6, 11]. A previous investigation [2] showed that after simultaneous bilateral ligation of the common carotid arteries in rats a disturbance of the cerebral circulation is observed in the mesencephalon, diencephalon, and in particular, in the cerebral hemispheres. Meanwhile in these parts of the brain the intensity of metabolism of total PL is depressed; the degree of depression correlates to a definite degree with the degree of disturbance of the cerebral circulation. Changes in the concentration of total PL has not been observed in any parts of the brain so far investigated.

However, the structural and functional heterogeneity of PL, known from the literature, demands that the effect of ischemia be studied on metabolism of the individual members of this group of substances. The only data available in the literature on this problem [9] describe a decrease in the concentration of both total PL and of the fractions of phosphatidylcholines (PCh) and phosphoinositides (PI) in brain tissue during ischemia.

The object of this investigation was to study the degree of depression of metabolism of individual PL fractions after simultaneous bilateral ligation of the common carotid arteries in the cerebral hemispheres and diencephalon, in which disturbances of the blood supply and of the intensity of metabolism of total PL are clearly present but differ in severity.

EXPERIMENTAL METHOD

The intensity of metabolism of the different PL fractions of brain tissue was determined as the rate of incorporation of radioactive phosphate into them. Radioactive $Na_2H^{32}PO_4$ was injected intraperitoneally in a dose of 5 μCi/g into adult male Wistar rats 30 min after ligation of both common carotid arteries. The brain was removed 60 min after injection of the isotope, blood vessels and meninges were carefully removed, blood was washed off with physiological saline, and the cerebral hemispheres and diencephalon were isolated. PL were extracted with a mixture of chloroform and methanol (2:1). A known volume of the washed lipid extract was used for analysis of total PL; the residue was evaporated to dryness in a current of nitrogen and dissolved in 0.8-1 ml chloroform for subsequent fractionation by thin-layer chromatography on silica-gel (Silikagel' L 5/40). Each sample was applied to the plate in strips 2-2.5 cm wide, with a load of 10-13 mg lipid phosphorus per strip. To separate the PL, systems of solvents modified by the writer previously and described in the literature [7] were used. Successive linear chromatographic fractionation was carried out with the following systems of solvents: chloroform–methanol–acetic acid–water (50:30:8:4) and chloroform–methanol–acetic acid–water–petroleum benzine (50:25:7:3:20). The plate was removed from the first mixture when the solvent front had reached 6.5 cm from the starting line (15 min) and

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TABLE 1. Intensity of Metabolism of Individual PL Fractions in Parts of Rat Brain Following Ligation of Common Carotid Arteries

Fraction	Indices	Cerebral hemispheres		Diencephalon	
		control	ischemia	control	ischemia
Total PL	n	18	21	16	22
	$M \pm m$	2,15±0,13	1,32±0,072	1,46±0,056	1,26±0,052
	%	100	61,4	100	86,3
	P	—	0,0004	—	0,019
PCh	n	14	23	17	25
	$M \pm m$	0,86±0,02	0,60±0,033	0,67±0,04	0,53±0,03
	%	100	69,8	100	79,1
	P	—	0,0002	—	0,009
PS	n	18	22	16	23
	$M \pm m$	0,50±0,041	0,31±0,032	0,32±0,046	0,35±0,033
	%	100	62,0	100	109,3
	P	—	0,004	—	0,64
PEA	n	18	22	18	22
	$M \pm m$	0,48±0,036	0,27±0,018	0,39±0,034	0,43±0,036
	%	100	56,3	100	110,2
	P	—	0,0005	—	0.24
PI	n	15	18	16	23
	$M \pm m$	14,58±0,85	8,75±0,49	9,91±0,67	8,45±0,41
	%	100	60,0	100	85,2
	P	—	0,0004	—	0,04
SPM	n	13	21	16	18
	$M \pm m$	0,43±0,029	0,36±0,036	0,29±0,039	0,30±0,038
	%	100	83,7	100	103,7
	P	—	0,14	—	0,82

transferred without drying to the second mixture. The total distance traveled by the mixture of solvents from the starting line was 17 cm and the total fractionation time in the two mixtures 75 min. To identify the spots, markers and corresponding specific reagents were used. The spots were developed in iodine vapor. The spots were removed from the plates by means of a water-jet pump on No. 3 glass filters. The PL were eluted from silica gel on the filters with a mixture (3 ml) of chloroform and methanol in different proportions: for PCh and sphingomyelin (SPM) 1:4, for PI 3:2, for phosphatidylserine (PS) and phosphatidylethanolamine (PEA) 4:1, and for the other PL 9:1, and from the starting line 2:1 [3]. All the fractions were then extracted twice with 3 ml of a chloroform–methanol–water mixture (65:35:8) [10] and, finally, with 3 ml of a methanol–acetic acid–water mixture (94:1:5) [8]. The PL phosphorus and inorganic phosphorus content of all samples (in μg phosphorus/g wet weight of tissue) was determined and the relative specific radioactivity (RSR) measured; the latter was taken as the intensity of PL metabolism and calculated as the ratio of the specific radioactivity (SR) of PL phosphorus to SR of inorganic phosphorus. The significance of the results was determined by Student's and Wilcoxon's criteria.

EXPERIMENTAL RESULTS

The intensity of metabolism of both total PL and its individual fractions in the cerebral hemispheres during the period of 30-90 min after ischemia for 90 min was sharply reduced; the decrease was statistically significant compared with the control ($P < 0.01$). The SPM fraction, which showed a tendency to decrease because of low values of RSR and the wide scatter of the data, did not differ significantly from the control and was the exception to the rule. In the diencephalon metabolism of total PL also was depressed, but on account of a statistically significant ($P < 0.05$) decrease in the intensity of metabolism of the PCh and PI fractions only. In the other fractions tested no significant changes of metabolism were observed (Table 1). No changes in the PL content were found in either part of the brain in the hypobaric or hypotoxic forms of hypoxia [1, 4]. In this respect the present results differ from those of Sobotka and Hinzen [9].

The experimental results show that in the cerebral hemispheres, where the degree of disturbance of the blood supply after ligation of the common carotid artery was maximal (up to

28.6% compared with the control) compared with that in other parts of the brain there was a significant decrease in the intensity of metabolism both of total PL and of its individual members, except in the case of SPM. No definite difference was found in the degree of depression of metabolism of the PCh, PI, PS, and PEA fraction. The similarity in the response of individual PL fractions to disturbance of the blood supply and the associated oxygen deficiency indicates to some degree that this factor influences certain common links in the chain of biosynthesis of individual members of the PL group and possibly, in particular, those connected with the participation of CTP as specific cofactor [6]. Similar results were obtained previously in the writer's laboratory in hypobaric and histotoxic forms of hypoxia, in which a clear decrease in the intensity of metabolism of both total PA and its individual fractions was observed in the cerebral hemispheres; in both these forms of hypoxia the decrease was roughly the same for all groups of PL tested [1, 4].

In the diencephalon, in which the intensity of metabolism of total PL and its individual fractions is normally lower than in the cerebral hemispheres and disturbance of the blood supply through ischemia is less marked (not exceeding 55.5% compared with the control), the observed depression of total PL metabolism is due to a decrease in the intensity of metabolism of the PCh and PI fractions in the diencephalon, the existing suggestion can be accepted that these fractions are linked, to a greater degree than the others, with the maintenance of the level of functional activity of the brain cells.

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