PHOSPHOLIPID METABOLISM IN MICROSOMES AND CYTOSOL OF BRAIN TISSUE OF NORMAL RATS AND RATS WITH HYPOXIC HYPOXIA

S. V. Gasteva, T. E. Raize, and L. M. Sharagina

UDC 612.8.015:547.953:612.273

The content and intensity of metabolism of phosphate groups of various phospholipids (phosphatidylcholines, monophosphoinositides, aminophospholipids) were studied in homogenate, microsomes, and cytosol of the rat brain under normal conditions and in hypoxic hypoxia (240 mm Hg). The concentration of phospholipids per milligram protein was found to be highest in the microsomes and lowest in the cytosol, but the total phospholipids of the cytosol had the highest metabolic rate of their free phosphate groups. Hypoxia, while not affecting the phospholipid concentration, depressed the intensity of their metabolism; this depression of metabolism, moreover, was about equal in all the tissue preparations studied.

KEY WORDS: *phospholipid metabolism; rat brain; hypoxia; subcellular fractions.*

Phospholipids (PL) are essential components of biomembranes. However, it is already generally recognized that not all cellular membrane structures can synthesize PL *de novo*. Whereas in the endoplasmic reticulum all PL can be synthesized, the mitochondria of various mammalian tissues do not possess the complete set of enzymes needed to synthesize most PL [10, 11, 13]. In mammalian cells PL are known to be transported from the site of their synthesis in the endoplasmic reticulum to other cell membranes through the aqueous phase of the cells with the participation of cytosol proteins [15]. Despite the extensive literature on the phospholipid composition of the various subcellular formations, the phospholipid composition of the hyaloplasm, in which this transport takes place, has received very little study. Moreover, there is very little information in the literature on changes in the PL metabolism of subcellular particles in various pathological states and, in particular, in hypoxia.

The object of the present investigation was to study the content and intensity of metabolism of PL phosphorus in the microsomes and cytosol of brain tissue of normal rats and rats exposed to hypoxic hypoxia.

EXPERIMENTAL METHOD

Adult Wistar albino rats were kept in a pressure chamber under a pressure of 240 mm Hg for 2 h. Orthophosphate-^{32}P (5 μCi/g body weight) was injected subcutaneously immediately before the animals were placed in the pressure chamber. The rats were decapitated 120 min after injection of isotope. A 10% homogenate of the cerebral hemispheres was prepared in 0.32 M sucrose in 0.01 M Tris-HCl, pH 7.4. According to the generally accepted scheme [3, 14], with a few modifications, two subcellular fractions were obtained: microsomes (centrifugation for 1 h at 100,000g) and cytosol. Lipids were extracted with a mixture of chloroform and methanol (1:2) by the method of Bligh and Dyer [8] from the homogenate, suspension of microsomes, and cytosol. The extract was washed with 0.29 M NaCl and lipids separated from it by thin-layer chromatography on silica-gel [2, 12] with some modifications. The following PL groups were obtained on the chromatograms (beginning from the start): sphingomyelin (SPM), phosphatidylcholine (PCh), monophosphoinositide (MPI), total aminophospholipids (APL — phosphatidylethanolamine and phosphatidylserine), and acid PL, evidently phosphatidic acids plus polyglycerophosphatides. After elution of the PL from the spots with mixtures of chloroform and methanol the content of lipid phosphorus was determined by

Laboratory of Regulation of Brain Metabolism, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 11, pp. 533-535, November, 1978. Original article submitted March 29, 1978.

0007-4888/78/8611-1439$07.50

TABLE 1. Content and Intensity of Metabolism of PL Phosphorus of Homogenate, Microsomes, and Cytosol from Brain of Normal Rats and Rats with Hypoxia (M ± m)

Tissue preparation	Statistical index	Total PL		PCh		MPI		APL	
		μgP/mg protein	RSR	% of total PL content	RSR	% of total PL content	RSR	% of total PL content	RSR
Normal									
Homogenate	*M*	17,7	2,31	38,0	2,05	2,72	31,7	50,6	0,79
	±*m*	1,1	0,14	1,24	0,19	0,28	1,83	1,6	0,12
Microsomes	*M*	28,2	3,32	43,7	2,49	3,85	48,4	42,9	1,52
	±*m*	1,0	0,18	2,74	0,19	0,55	5,0	2,22	0,08
Cytosol	*M*	3,24	4,58	43,8	3,10	5,06	50,0	31,0	1,66
	±*m*	0,44	0,26	2,69	0,31	0,81	4,11	2,32	0,17
Hypoxia									
Homogenate	*M*	18,0	1,58	38,9	1,18	3,07	24,0	47,7	0,51
	+*m*	0,47	0,07	1,27	0,14	0,12	1,61	1,50	0,05
	Percent of normal	102	68	102	58	113	76	94	65
	P	0,1	0,001	0,1	0,01	0,1	0,02	0,1	0,05
Microsomes	*M*	26,9	2,56	40,2	1,66	3,91	33,5	47,8	0,88
	±*m*	0,79	0,14	1,68	0,12	0,24	1,8	1,37	0,12
	Percent of normal	95	77	92	67	101	69	111	58
	P	0,1	0,01	0,1	0,01	0,1	0,02	0,05	0,001
Cytosol	*M*	3,59	2,97	51,5	2,19	3,17	26,7	33,4	1,13
	±*m*	0,24	0,24	1,93	0,16	0,70	4,11	0,64	0,10
	Percent of normal	111	65	117	71	63	53	107	68
	P	0,1	0,001	0,05	0,05	0,1	0,01	0,1	0,05

Bartlett's method [7] and its radioactivity (with the Izokap-300 scintillation counter), and the specific radioactivity (SR) of the lipid phosphorus was calculated in counts/μg phosphorus/min. The relative SR (RSR), calculated as the ratio of SR of PL phosphorus to SR of inorganic phosphorus (times 100), was used as the measure of intensity of PL phosphorus metabolism. The total PL content in each subcellular fraction was expressed in micrograms phosphorus per gram wet weight of tissue or per milligram proteins. The content of individual PL was expressed as percentages of the total PL content extracted from all the spots. Protein was determined by Lowry's method [9]. The number of experiments of each variant ranged from 6 to 13.

EXPERIMENTAL RESULTS

The total PL content, in μg phosphorus/g weight of tissue, was 1820 from homogenate, 271 for microsomes (15% of the PL content in the homogenate), and 63 for cytosol (3% of the content in the homogenate), in agreement with data in the literature [6]. The protein content in homogenate, microsomes, and cytosol was 88.4, 9.8, and 19.5 mg protein/g wet weight of tissue, in agreement with the results obtained by Palladin et al. [4]. The higher PL content in the microsomes than in the cytosol will be clear from Table 1; the PL/protein ratio in the cytosol was almost an order of magnitude lower than in the microsomes. The distribution of individual PL in the homogenate and in the fractions investigated was about the same, but the main components of PL were PCh and APL, although in the cytosol the proportion of APL was lower than in the homogenate and microsomes ($P < 0.001$). Results only for PCh, MPO, and APL are given in Table 1, for the data on SPM and acid PL displayed considerable scatter. The results obtained for the content (3-5% for SPM and 1-3% for acid PL) and for RSR (0.3-0.7 for SPM and 3-5 for acid PL) were of the same order of magnitude as the results given for homogenate by Dvorkin et al. [1]. The intensity of metabolism of total PL in the cytosol was significantly higher (by 1.4 times) than in the microsomes. As regards the individual PL groups, no significant differences were observed between the values of RSR for the two subcellular fractions, but they were considerably higher than the corresponding values of RSR for the homogenate. The difference between RSR of total PL for these two subcellular fractions must evidently be attributed to other PL groups than those which were studied.

Hypoxia had virtually no effect on the PL content in the homogenate or in either of the two fractions tested; moreover, as Table 1 shows, this is true of both total PL and individual PL groups, a decrease was observed in RSR of PL phosphorus in hypoxia. The degree of

depression of metabolism of total PL in hypoxia was evidently greater in the cytosol than in the microsomes, although differences in the reaction were on the border line of significance ($P = 0.05$). Whereas an equal decrease in RSR during hypoxia in both subcellular fractions tested was characteristic of PCh and APL, the decrease in RSR for MPI was much greater in the cytosol than in the microsomes.

The quantitatively similar degree of depression of metabolism observed previously in the writers' laboratory both for total PL and for their individual fractions under analogous conditions of hypobaric hypoxia [5] was thus confirmed in this study of the subcellular fractions of brain tissue. The relatively higher metabolic activity of total PL and PCh of the cytosol of rat brain tissue than of the microsomes may be evidence that two PL pools are synthesized in the endoplasmic reticulum, one with lower RSR for renewal of the membranes of the endoplasmic reticulum themselves, the other with a higher rate of synthesis of PL which are then transported to other membranous structures through the aqueous phase of the nerve cells.

LITERATURE CITED

1. V. Ya. Dvorkin, D. A. Chetverikov and A. A. Shmelev, Ukr. Biokhim. Zh., 37, 529 (1965).
2. G. V. Kiselev, Biokhimiya, 34, 483 (1969).
3. E. P. Kreps, "Phospholipids of cell membranes of the nervous system in the development of the animal kingdom" (The 22nd Bakh Lecture) [in Russian], Leningrad (1967).
4. A. V. Palladin, Ya. V. Belik, and N. M. Polyakova, The Brain Proteins and Their Metabolism [in Russian], Kiev (1972).
5. D. A. Chetverikov, S. V. Gasteva, V. Ya. Dvorkin, et al., in: Proceedings of the 5th All-Union Conference on Neurochemistry [in Russian], Tbilisi (1970), pp. 274-284.
6. G. V. Ansell, in: Form and Function of Phospholipids, (edited by G. V. Ansell et al.), Vol. 3, Amsterdam (1973), pp. 377-422.
7. G. R. Bartlett, J. Biol. Chem., 234, 466 (1959).
8. E. G. Bligh and W. I. Dyer, Can. J. Biochem., 37, 911 (1959).
9. O. H. Lowry et al., J. Biol. Chem., 193, 265 (1951).
10. W. C. McMurray, Can. J. Biochem., 53, 784 (1975).
11. E. K. Miller and R. M. C. Dawson, Biochem. J., 126, 805 (1972).
12. V. P. Skipski, R. F. Peterson, J. Sanders, et al., J. Lipid Res., 4, 227 (1963).
13. L. M. G. Van Golde, J. Raben, J. J. Batenburg, et al., Biochim. Biophys. Acta, 360, 179 (1974).
14. V. P. Whittaker, Prog. Biophys. Molec. Biol., 15, 39 (1965).
15. K. W. A. Wirtz, Biochim. Biophys. Acta, 344, 95 (1974).