PHOSPHOLIPIDS OF SUBCELLULAR FRACTIONS OF RAT BRAIN AND LIVER TISSUE IN HYPOXIC HYPOXIA

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The composition and metabolism of membrane phospholipids (PL) largely depend on the type of cells and tissues [3]. This conclusion follows particularly clearly from a comparison of PL metabolism in liver and brain tissues. Whereas in the brain intracellular PL metabolism is exclusively concerned with maintenance of structural elements in cells of brain tissue itself, in the liver PL metabolism also includes an additional function specific for the liver, namely replenishment of the plasma lipoprotein reserves. The results of numerous investigations into the composition and metabolism of PL in subcellular structures [4, 7-9] and in the liver [5, 10, 11, 13] suggests that comparison of the effect of certain pathogenic factors on PL metabolism in subcellular particles of these tissues is important.

The aim of this investigation was to compare the content and intensity of metabolism of PL in subcellular structures of rat liver and brain tissue and to compare the effects of hypoxic hypoxia, of different degrees of severity, of these parameters.

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar albino rats, receiving an intraperitoneal injection of [^{32}P]orthophosphate in a dose of 0.2 GBq/kg body weight. General hypoxia was created by placing the rats in a pressure chamber under a pressure of 240 mm Hg (moderately severe hypoxia) or 200 mm Hg or lower, so that the mortality among the animals was about 50% (severe hypoxia). The rats were placed in a pressure chamber immediately after injection of the isotope. Exactly 2 h after this injection the control and hypoxic rats were decapitated and the cerebral hemispheres removed, washed carefully to remove blood, and stripped of membranes of the vascular plexus. The liver was removed and perfused with cold physiological saline. All procedures were carried out on ice and as quickly as possible. Subcellular fractionation of brain tissue followed the usual scheme [2, 14]. The homogenate, microsomes (100,000g, 1 h), cytosol, and mitochondria obtained by centrifugation of the unpurified mitochondrial fractions in a sucrose density gradient (53,700g, 2 h) were investigated. Subcellular fraction of the liver tissue was carried out by the method in [12]. All procedures relating to extraction of the lipids, washing to remove nonlipid impurities, and determination of the content and metabolism of PL followed techniques described previously [1]. The measure of intensity of phosphorus metabolism of PL was the relative specific radioactivity (RSR), calculated as the ratio between specific radioactivity (SR) of PL phosphorus and SR of inorganic phosphorus of the homogenate, multiplied by 100. The PL content was expressed in micrograms of PL phosphorus/mg protein. The results were subjected to statistical analysis by the Student–Fisher method. The number of experiments in each variant varied from six to 24.

EXPERIMENTAL RESULTS

Data on the content and intensity of metabolism of PL phosphorus in the structures studied under normal conditions are given in Table 1. Brain tissue was richer in PL than liver tissue. This was apparent both in the homogenate, where the PL/protein ratio was almost 3 times higher in brain than in liver tissue, and at the subcellular level, where this ratio was 2-4 times higher for brain than for liver. Unlike the content of PL, the intensity of its metabolism was higher in liver tissue than in brain tissue, by 2 to 4 times depending on the structure studied. All these results are in agreement with data in the literature.

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TABLE 1. Content and RSR of PL Phosphorus in Subcellular Structures of Normal Rat Brain and Liver (M ± m)

Tissue preparation	Brain		Liver	
	μg P/mg protein	RSR	μg P/mg protein	RSR
Homogenate	18,6 ± 0,9	2,26 ± 0,10	6,43 ± 0,33	9,96 ± 0,54
Microsomes	28,6 ± 1,1	2,44 ± 0,17	14,0 ± 1,3	10,3 ± 0,57
Cytosol	3,64 ± 0,51	4,18 ± 0,34	0,92 ± 0,05	10,4 ± 0,46
Mitochondria	15,8 ± 0,83	4,28 ± 0,42	7,56 ± 0,48	8,21 ± 0,50

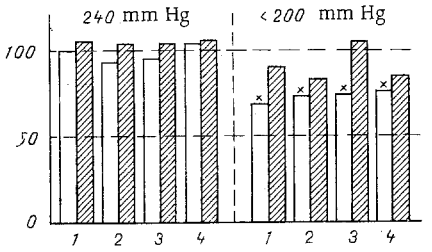

Fig. 1. PL content (in % of control) in subcellular fractions of brain and liver tissues of rats with hypoxic hypoxia of varied severity. 1) Homogenate, 2) microsomes, 3) cytosol, 4) mitochondria. Unshaded columns – brain tissue, shaded columns – liver. *P < 0.05 compared with control.

In the liver the highest RSR of PL phosphorus was observed, incidentally, in the microsomes. This is in agreement with data in the literature [10] and it confirms the fact that biosynthesis of cellular PL is located mainly in the endoplasmic reticulum, from which they are subsequently transported to other hepatocyte membranes. In brain tissue, the highest RSR was observed, not in the microsomes, but in mitochondria. As we pointed out previously [7], this phenomenon can evidently be explained by the fact that the pool of PL in the endoplasmic reticulum of brain tissue, synthesized to replace the membranes of the endoplasmic reticulum itself, has a lower RSR than the pool of PL which are later transported to other membrane structures through the cytoplasm.

Under normal conditions, therefore, brain and liver tissues differ in their PL content, the intensity of their PL metabolism, and the ratio between the rates of their metabolism in different subcellular fractions.

Data showing the effect of two degrees of hypoxia on the PL content in homogenate and subcellular fractions of brain and liver tissues are given in Fig. 1. They show that moderately severe hypoxia (240 mm Hg) does not affect the PL content in any of the fractions of these tissues tested. More severe hypoxia (<200 mm Hg) caused no statistically significant changes in the PL content in the liver structures studied. By contrast, this severe hypoxia caused a significant decrease in the PL content in homogenate (by 35%), microsomes (by 27%), and mitochondria (by 24%) from the brain. The decrease in the cytosol was not statistically significant, evidently because the values obtained for its PL content were very small and had considerable scatter. Data on the effect of hypoxia on RSR of PL phosphorus in the preparations studied show that moderately severe hypoxia caused virtually no change in the intensity of PL metabolism in liver tissue (Fig. 2). In brain tissue under these conditions a decrease in RSR was observed, and it was particularly marked in the homogenate and mitochondria. The intensity of PL metabolism in the liver showed a decrease only in the severer degree of hypoxia.

Comparison of the effect of hypoxia on PL metabolism in brain and liver tissues thus showed that differences in the sensitivity of these two tissues to hypoxia are manifested not only at the homogenate level, as was shown previously [6], but also at the subcellular level. Moderately severe hypoxia (240 mm Hg), which reduced the intensity of brain PL metabolism, had no effect on either the content or the intensity of metabolism of PL in subcellular liver fractions. More severe hypoxia (<200 mm Hg) caused a significant fall in RSR of PL phosphorus in the liver, but without any effect on the PL content in any of the subcellular fractions studied. Besides

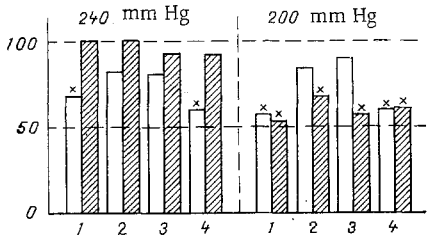

Fig. 2. RSR of PL phosphorus (in % of control) in cellular fractions of brain and liver tissue of rats with hypoxic hypoxia of varied severity. Legend as to Fig. 1.

a decrease in RSR of PL, a decrease in their content also was observed in brain tissue in severe hypoxia. The view that the brain is more sensitive to hypoxia than other tissues of the body and, in particular, than the liver is thus confirmed by the example of PL metabolism in subcellular structures.

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