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CHANGES IN THE PHOSPHOLIPID SPECTRUM AND ACTIVITY OF SOME ENZYME SYSTEMS OF PHOSPHOLIPID SYNTHESIS IN THE BRAIN AND LIVER OF ALBINO RATS WITH ALLOXAN DIABETES

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KEY WORDS: phospholipids; rat brain and liver; alloxan diabetes.

The physiological role of phospholipids (PL) as essential components of biological systems in living organisms is evident. PL are known to participate in the regulation of activity of membrane-bound enzymes [12], of insulin secretion [5], limitation of glycolytic reactions in the brain [9], the adjustment of tissue sensitivity to the action of hormones [8], and compensation of a deficiency of the main energy substrate – glucose [2].

In the investigation described below changes in the qualitative and quantitative composition of PL in brain and liver tissue were studied in albino rats with severe metabolic disturbances associated with alloxan diabetes, in the course of conversions of certain products of lipogenesis – free glycerol and L-α-glycerophosphate (GP), and of the activity of the corresponding enzyme systems – glycerokinase (GK), L-α-glycerophosphate dehydrogenase (GPD) in NAD-dependent (GPD-1) and NADH-dependent (GPD-2) systems.

EXPERIMENTAL METHOD

Diabetes was induced in noninbred albino rats of both sexes weighing 170-200 g by intraperitoneal injection of alloxan in a dose of 15 mg/100 g body weight. Animals with a blood glucose higher than 180 mg% were killed on the 20th day of the disease, and chloroform-methanol extracts of PL from acetone powders of brain and liver were fractionated by linear ascending chromatography on FN-11-Filtrak (East Germany) paper, soaked in silicic acid [10]. The quantity of free glycerol [4], activity of GK and GPD-1, and the GP level were determined in the fraction obtained at 1700g by a microspectrophotometric method [7], GPD-2 activity was determined as in [6], and the blood glucose was estimated by the orthotoluidine method. A mixture of phosphotrioses was obtained by Meyerhof's method [11].

EXPERIMENTAL RESULTS

An increase in the total acid PL (APL) by about 62% was found in the brain tissue of albino rats on the 20th day of alloxan diabetes, accompanied by a relatively stable level of total and neutral PL (NPL), mainly on account of a twofold increase (by 121%) in the content of cardiolipins (CL), which play an important role in regulation of the activity of respiratory chain enzymes [3]. The possibility cannot be ruled out that the observed increase in the CL level may be a compensatory-adaptive reaction of the body due to inhibition of

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TABLE 1. Changes in Content of Individual and Total Phospholipids (in μg lipid phosphorus/g wet weight of tissue) in Brain and Liver of Rats with Alloxan Diabetes (M ± m)

Index	Brain			Liver		
	control	diabetes	changes, percent of control	control	diabetes	changes, percent of control
Monophosphinositides	99,0±4,0	157,0±11,0 $P<0,001$	159	108,0±5,0	104,0±10,0	96
Phospholipid-sulfatide fraction	66,2±3,1	118,3±4,8 $P<0,001$	179			—
Lysophosphatidylcholines	—	—	—	61,2±4,0	121,6±15,8 $P<0,005$	199
Sphingomyelins	224,8±9,8	265,8±19,4	118	101,0±6,0	180,2±21,7 $P<0,005$	179
Phosphatidylcholines	750,2±17,0	568,3±47,6 $P<0,001$	76	459,4±11,2	327,1±22,1 $P<0,001$	71
Phosphatidylserines	205,1±7,9	279,6±32,6 $P<0,05$	136	73,8±2,6	112,8±8,8 $P<0,001$	153
Phosphatidylethanolamines	348,7±13,4	384,5±32,5	110	215,0±5,7	155,3±14,0 $P<0,005$	72
CL	113,6±6,7	251,4±5,9 $P<0,001$	221	74,1±5,7	130,1±16,5 $P<0,005$	176
Total NPL	1323,7±22,0	1218,8±59,4	92	835,9±12,1	784,2±68,6	94
Total APL	483,9±12,0	782,6±38,6 $P<0,001$	162	256,2±5,5	346,4±30,7 $P<0,025$	135
Ratio NPL/APL	2,8±0,1	1,5±0,1 $P<0,001$	55	3,2±0,1	2,3±0,1 $P<0,001$	69
Grand total of PL	1803,0±25,4	2025,5±95,0 $P<0,05$	112	1092,0±16,1	1118,1±90,3	102

TABLE 2. Changes in Content of Free Glycerol (in mg/g wet weight of tissue), L-α-glycerophosphate (GP), and GK, GPD-1, and GPD-2 Activity (in μmoles NADH/g wet weight of tissue) in Brain and Liver of Rats with Alloxan Diabetes (M ± m)

Index	Brain			Liver		
	control	diabetes	changes, percent of control	control	diabetes	changes, percent of control
Glycerol	1,36±0,05	1,56±0,11	114,7	2,21±0,05	2,56±0,12 $P<0,01$	115,8
GP	0,58±0,06	0,34±0,02 $P<0,001$	58,6	1,21±0,09	1,43±0,11	118,2
GK	0,51±0,03	0,35±0,02 $P<0,001$	68,6	0,98±0,08	0,33±0,01 $P<0,001$	33,7
GPD-1	2,88±0,08	1,37±0,07 $P<0,001$	47,6	8,03±0,47	11,25±0,54 $P<0,001$	140,1
GPD-2	3,22±0,27	2,82±0,19	85,7	9,22±0,24	13,62±0,88 $P<0,001$	147,2

activity of the above-mentioned enzyme systems in this type of pathology. Similar changes also were found in the content of monophosphoinositides, the phospholipid-sulfatide fraction, and phosphatidylserine, the levels of which rose by 59, 79, and 36% respectively.

A considerable change in the PL spectrum also was observed in the liver tissue of the rats with alloxan diabetes. In tissue studied these changes were accompanied in particular by an increase in the CL content by 76%, accompanied by the formation of new molecular forms of these compounds, differing in their fatty acid composition [1].

In the writers' view, the most important changes in alloxan diabetes were an increase in the lysophosphatidylcholines by 99%, accompanied by a parallel decrease in the content of phosphatidylcholine by 29%, which could be explained by activation of the corresponding phospholipases. Of the other choline-containing PL, an increase in the content of sphingomyelins, which play the role of coenzyme in the transformation of free energy into bound energy of ATP, by 79% in the liver tissue also is noteworthy.

In alloxan diabetes destabilization of the ratio of total NPL to total APL was observed in the brain and liver tissue, evidence of the depth of the metabolic disturbances which developed, especially in lipid metabolism. In the study of the state of the enzyme system and intermediate products of phosphatide synthesis in these tissues, marked inhibition of GK activity was observed, especially in the liver – by 31 and 66% respectively. In all probability this change is directly related to inhibition of glycolytic reactions connected with disturbance of the utilization of glucose by the tissues in diabetes mellitus. Hence the ATP/ADP ratio was reduced on account of fall in the ATP level, with the result that activity of ATP-dependent GK was weakened.

Besides inhibition of GK activity, activity of GPD-1 and GPD-2 in the brain tissue was inhibited by 52 and 14% respectively. Depression of GK and GPD-2 activity in the brain was accompanied by a marked fall in the brain GP reserves. Under these circumstances the decrease in GPD-1 activity was assessed as compensatory preservation of the reserves of GP – the original compound in reactions of phosphatide synthesis.

An increase in GPD-1 activity (by 40%) in the liver tissue in all probability was dependent on intensification of the processes of gluconeogenesis in that organ in diabetes, whereas activation of GPD-2 was evidently directed toward replenishing the GP reserves in response to the inhibited state of GK.

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HYDROPEROXIDES OF FATTY ACIDS, FLUORESCENT PRODUCTS, AND TOCOPHEROL CONCENTRATIONS IN TISSUES OF RABBITS AFTER EXPOSURE TO COLD

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KEY WORDS: hydroperoxides of fatty acids; fluorescent products; tocopherol; peroxidation of lipids; liver; lungs; erythrocytes.

The study of the molecular mechanisms of the harmful action of cold on biological tissues is an essential condition for the understanding of the etiology and pathogenesis of diseases arising during or made worse by the action of cold.

Being an essential component of practically every form of stress, including cold stress, hydroperoxides of fatty acids formed during reactions of free-radical oxidation (FRO) of lipids, may act as agents disturbing the molecular organization of cell membranes [9] and may contribute to the onset of pathological changes. An indicator of such disturbances is the appearance in the tissues of fluorescent compounds, polymerization products of a protein–lipid complex [2]. The appearance of fluorescent compounds reflects profound structural and functional disturbances of cell membranes and of oxidative processes in the cells and tissues as a whole.

The object of this investigation was to study the possible role of FRO reactions of lipids in the development of destructive processes in the lungs of rabbits exposed to low temperatures.

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