PHOSPHOLIPID COMPOSITION OF RABBIT BRAIN SYNAPTOSOMES UNDER NORMAL CONDITIONS AND IN POISONING BY ORGANOPHOSPHORUS CHOLINESTERASE INHIBITORS

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The content of individual phospholipid fractions was studied in homogenates and synaptosomes from the brain of normal rabbits and rabbits poisoned with a lethal dose of an organophosphorus cholinesterase inhibitor belonging to the alkylthiophosphonate series. In normal rabbit brain synaptosomes the relative content of diacyl aminophospholipids (APL) was lower and the content of plasmalogen APL, phosphoinositides, and phosphatidylcholines was higher than in homogenates of the whole brain. Poisoning with the organophosphorus inhibitor reduced the content of diacyl APL by 25% and increased the content of plasmalogen APL by 17% in whole brain homogenates, but caused no change in the content of all phospholipid fractions tested in the brain tissue synaptosomes.

Key words: brain phospholipids; nerve endings; cholinesterase; poisoning by organophosphorus inhibitors.

Recent investigations have shown that the role of phospholipids (PL) in nerve tissue is not confined to that of building material for the membranous structures. Brain function and, in particular, membrane transport of the cations required for conduction and transmission of the nervous impulse [2] are essentially dependent on PL [9, 11, 14].

It was decided to study whether changes take place in the qualitative and quantitative composition of PL in nerve tissue in general and in the synaptosomes in particular during disorders of synaptic transmission arising through poisoning by organophosphorus cholinesterase inhibitors (OPI).

EXPERIMENTAL METHOD

Male rabbits weighing 2.5 g received a lethal dose (10 mg/kg) of an OPI – preparation GA-3 – a thiophosphonic acid derivative: O-n-butyl-S-n-butylmethylthiophosphonate, synthesized by N. N. Godovikov and A. A. Abduvakhabov [1], by intramuscular injection. The animals were decapitated 30-40 min after injection of the OPI, when definite signs of severe poisoning (salivation, tremor, spasms) had developed. Synaptosomes were isolated by de Robertis' method [15] in the modification of Kreps et al. [6] from a homogenate of whole brain [8].

Lipids were extracted from the synaptosomal fraction and the brain homogenate by the method of Folch et al. [12] in Suzuki's modification [19]. The chloroform-methanol extract was washed with 0.75% KCl in the proportion of 0.2 ml to 1 ml extract in order to remove gangliosides and other water-soluble impurities, after which the bottom phase was rewashed with a mixture of chloroform, methanol, and KCl solution (3:47:48).

The PL of the homogenates and synaptosomes were fractionated by chromatography on silica gel columns by the scheme described earlier [4]. The following PL fractions were isolated and investigated:

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TABLE 1. Phospholipid Composition of Homogenates and Synaptosomal Fraction of Rabbit Brain Tissue under Normal Conditions and during Poisoning with the Compound GA-3 (in % of total lipid phosphorus; M ± m)

PL fractions	Homogenate			Synaptosomes		
	control	preparation GA-3	P	control	preparation GA-3	P
PA + PGP	4,6± 0,3	4,6± 0,2	>0,1	4,6± 0,2	4,6± 0,2	>0,1
APL						
diacyl	18,9± 0,8	14,3± 0,6	<0,01	7,9± 0,9	7,1± 0,5	>0,1
plasmalogen	23,3± 1,5	28,1± 1,5	<0,05	27,4± 0,9	28,2± 2,7	>0,1
PI	9,6± 0,1	9,9± 0,6	>0,1	11,7± 1,2	9,3± 0,5	>0,1
PC	29,4± 1,1	29,3± 1,5	>0,1	34,7± 0,6	35,1± 1,2	>0,1
SPM	11,2± 0,4	12,0± 0,4	>0,1	10,8± 0,5	9,9± 0,4	>0,1

Legend: PA + PGP) phosphatidic acids + polyglycerophosphatides; APL) aminophospholipids; PI) phosphoinositides; PC) phosphatidylcholines (lecithins); SPM) sphingomyelins.

phosphatidic acids and polyglycerophosphatides, aminophospholipids (APL), which were divided into diacyl and plasmalogen forms, phosphatidylcholines, phosphoinositides, and sphingomyelins. The content of each PL fraction was calculated from the content of lipid phosphorus after mineralization of the samples with sulfuric and nitric acids and was expressed as a percentage of the phosphorus of the total PL.

EXPERIMENTAL RESULTS AND DISCUSSION

Comparison of the phospholipid composition of the homogenates and synaptosomes of normal rabbit brain (Table 1) shows, first, that the synaptosomes contain the same phospholipid fractions as are present in the homogenate. This is in full agreement with the results obtained by other workers who showed that PL of the synaptosomes do not differ qualitatively in principle from PL of the homogenate and other membranous structures of nerve tissue [5, 16]. Meanwhile definite differences were found in the quantitative content of the individual PL fractions: content of diacyl forms of APL in the synaptosomes was considerably lower, while that of the plasmalogen forms of APL, phosphoinositides, and phosphatidylcholines was higher than in the whole brain homogenate. As regards the enrichment of the synaptosomes with phosphatidylcholines and phosphoinositides, the results agree with data in the literature [5, 16] and can evidently be explained by the participation of precisely these PL in metabolic conversions in the acetylcholine system during conduction of the nervous impulse. The increased content of the plasmalogen forms of APL and, conversely, the reduced content of diacyl forms revealed by these experiments were somewhat unexpected; as regards the relative content of plasmalogens (27.4% of the total PL content) the synaptosomes resembled the fraction of myelin fragments, in which plasmalogens, especially ethanolamine-plasmalogens, occupy the foremost place ahead of all the other forms of PL [18]. The results of the present experiments emphasize an important feature distinguishing the intracellular distribution of plasmalogens in brain tissue: they are present selectively in increased amounts in subcellular fractions and they are specific for nerve tissue, in which they are present in myelin and synaptosomes.

In OPI poisoning the only significant changes were those in the quantitative ratio between the diacyl and plasmalogen forms of APL in the rabbit brain homogenate: a decrease of 25% in the content of diacyl forms and an increase of 17% in the content of plasmalogen forms of APL (Table 1). The fact that the content of all the PL fractions tested remained unchanged in the synaptosomes was unexpected; consequently, the changes in the content of diacyl and plasmalogen forms of APL discovered in OPI poisoning in the brain homogenates were due to changes in the content of these PL in some other subcellular particles of brain tissue, possibly in the glial cells, but not in the synaptosomes, as might have been expected having regard to the specific nature of the experimental procedure.

These facts are further confirmation of the view expressed previously by the writers regarding the nonspecific character of the effect of OPI poisoning on brain PL metabolism [7]. For instance, a small decrease in the total PL content in the rabbit brain was observed not only in convulsions evoked by complete inhibition of cholinesterase by the action of the OPI, but also in convulsions caused by picrotoxin, a

substance with no anticholinesterase action [7]. Furthermore, the decrease in the intensity of PL metabolism in the mouse brain under the influence of OPI is only the result of the hypothermia accompanying the poisoning. In cases in which hypothermia did not develop, OPI did not affect the intensity of PL metabolism in the brain [3]. Meanwhile, in various pathological disturbances in nerve tissue leading to demyelinization, changes in the phospholipid composition take place chiefly on account of the components of the ethanolamine-containing PL; either the fatty-acid composition of the PL is modified or the relative quantitative proportions of the individual PL components are altered [13, 17, 21].

The reciprocal character of the changes in the content of diacyl and plasmalogen forms of APL discovered in these experiments in the rabbit brain during OPI poisoning evidently points to the possible interconversion of these two forms of APL; this hypothesis is in agreement with existing views regarding the biosynthesis of tissue plasmalogens by the dehydrogenation of the corresponding alkyl-acyl forms of PL [10, 20].

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