EFFECT OF ORGANOPHOSPHORUS CHOLINESTERASE INHIBITORS ON METABOLISM OF INDIVIDUAL PHOSPHOLIPID FRACTIONS IN THE RAT BRAIN

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Poisoning with the organophosphorus cholinesterase inhibitor LG-63 in a sublethal dose (5 mg/kg) had no significant effect on the intensity of metabolism of the phosphate groups of the phospholipid fractions in the rat brain but led to significant changes in the content of the polyphosphoinositide fractions: the content of triphosphoinositides was reduced by 8% while that of diphosphoinositides was increased by 14%; the content of the remaining phospholipid fractions studied was unchanged. The changes found in the polyphosphoinositide content in the rat brain during poisoning by the cholinesterase inhibitor are regarded as a metabolic response of the brain tissue to the hypoxia which develops during poisoning by this inhibitor.

The authors have shown previously that poisoning by organophosphorus cholinesterase inhibitors (OPI) did not affect the content of total phospholipids (PL) in the cerebral hemispheres of rats, and changes in the intensity of metabolism of the phosphate group of total brain PL were observed only if OPI poisoning in the animals was accompanied by a sufficiently marked fall of body temperature [5, 6]. The study of total PL cannot give a complete answer to the question of the effect of OPI poisoning on PL metabolism in the brain, in particular because when the tissue is extracted with a neutral mixture of chloroform and methanol the diphosphoinositides (DPI) and triphosphoinositides (TPI; collectively poly-PI) do not pass into the extract. Yet it is these latter compounds which react most distinctly to changes in the functional state or metabolism of the brain tissue [3, 4].

The object of this investigation was to study the effect of poisoning by LG-63 on the content and intensity of metabolism of the phosphate group of individual PL fractions, including individual phosphoinositides, in the rat brain.

EXPERIMENTAL

Adult male Wistar rats were given in intramuscular injection of the compound LG-63 (O-ethyl-S-hexylmethylthiophosphanate) in a sublethal dose. After 30 min, when the signs of poisoning (spasms, salivation, blood-stained tears) were well marked, the animals were given a subcutaneous injection of radioactive phosphate ($Na_2HP^{32}O_4$) solution in a dose of 5 μCi/g body weight. Two hours after injection of the isotope the animals were decapitated and the cerebral hemispheres quickly removed and washed in cold physiological saline to remove blood. Lipids were extracted with a neutral mixture of chloroform and methanol (2:1) by Folch's method, and the resulting lipid extracts, after washing to remove radioactive inorganic phosphate and nonlipic contaminants with 0.29 M NaCl, were fractionated on a silica-gel column as described previously [7], after which some PL fractions were separated by mild alkaline hydrolysis by Dawson's method [8]. The following PL fractions were isolated from the neutral lipid extract of rat brain tissue and investigated: phosphatidic acids + polyglycerophosphatides (PA + PGP), diacyl aminophospholipids (diacyl APL), plasmalogen aminophospholipids (Plasm. APL), phosphatidyl cholines (PC), and

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TABLE 1. Relative Specific Radioactivity and Content of Individual Phospholipid Fractions in the Brain of Rats Poisoned with LG-63

Fractions of PL	Index	Relative specific radioactivity		Content (in μgP/g moist tissue)	
		control	LG-63	control	LG-63
Total PL	$-n$	9	13	10	14
	$M \pm m$	1,98±0,06	1,96±0,05 (99,2)	1911±26	1929±24,8 (100,5)
	P		>0,1		>0,1
PA + PGP	$-n$	10	14	9	13
	$M \pm m$	6,04±0,15	5,91±0,28 (96,3)	65,8±2,1	70,1±2,3 (106,5)
	P		>0,1		>0,1
Diacyl APL	$-n$	10	12	9	13
	$M \pm m$	1,02±0,05	0,97±0,03 (95,0)	514,6±15,1	501,8±12,2 (98,0)
	P		>0,1		>0,1
Plasm. APL	$-n$	9	14	10	14
	$M \pm m$	0,36±0,02	0,33±0,02 (92,0)	389,3±8,2	382,1±7,6 (98,2)
	P		>0,1		>0,1
PC	$-n$	10	13	7	7
	$M \pm m$	1,25±0,05	1,20±0,04 (96,2)	607,6±24,4	646,7±16,8 (106,1)
	P		>0,1		>0,1
SM	$-n$	8	7	9	12
	$M \pm m$	0,29±0,01	0,30±0,02 (103,5)	130,7±7,5	122,5±4,1 (94,0)
	P		<0,1		>0,1
MPI	$-n$	16	20	9	20
	$M \pm m$	18,7±0,6	19,0±0,6 (101,5)	57,4±1,6	58,7±1,0 (102,5)
	P		>0,1		>0,1
DPI	$-n$	16	20	15	20
	$M \pm m$	29,1±0,8	31,3±1,4 (107,5)	19,1±0,6	21,7±0,7 (114,1)
	P		>0,1		<0,01
TPI	$-n$	13	19	16	20
	$M \pm m$	33,3±0,7	33,0±1,5 (100,0)	31,4±0,6	28,9±1,0 (92,0)
	P		>0,1		<0,05

Note: Figure in parentheses indicates percentage of control.

sphingomyelins (SM). To obtain and analyze individual PI fractions, the lipids were extracted from moist tissue with an acidified mixture of chloroform-methanol–concentrated HCl (200 : 100 : 1). The PI were separated into individual fractions (MPI, DPI, and TPI) by chromatography on paper treated with formalin [2]. The content of lipid phosphorus (in micrograms P of the fraction per gram moist tissue) and radioactivity were determined in each of the investigated PL fractions from the brain of the experimental and control rats. The intensity of metabolism of the individual PL fractions was judged from the relative specific radioactivity (RSR), which is the ratio between the specific radioactivity of the phosphorus in each PL fraction and the specific radioactivity of the inorganic phosphate of the brain tissue, multiplied by 100.

EXPERIMENTAL RESULTS

As Table 1 shows, poisoning by the cholinesterase OPI did not lead to changes in the intensity of metabolism of the phosphate groups in any of the investigated PL fractions of rat brain tissue. This was evidently because of the comparatively slight decrease in body temperature of the rats. This decrease ranged from 1 to 4.4°C, with a mean value of 2.4°C. As was shown previously, a significant decrease in the intensity of PL metabolism in the brain tissue occurred only when OPI poisoning was accompanied by a more marked decrease in the body temperature of the rats [5].

The investigated PL fractions in the rat brain can be divided into two groups. The first group contained PL fractions extractable with a neutral mixture of chloroform and methanol (PA + PGP, diacyl APL, plasm. APL, PC, SM), and MPI; their content was equal in the brain of the experimental and control rats. These results are in agreement with those of previous investigations in which no changes were observed in the content of PL extractable from the brain tissue by a neutral mixture of organic solvents after exposure

of the animals to various factors (acute hypoxia, acute radiation sickness, insulin hypoglycemia, etc.). The second group of PL fractions consists of poly-PI (DPI and TPI); their content showed definite changes in the brain tissue of the experimental rats: a decrease in the TPI and increase in the DPI content.

In OPI poisoning, just as during exposure to the other factors studied [3, 4], DPI and TPI were thus the most labile members of the PL group in the brain tissue. The opposite nature of the changes in the content of the poly-PI components in the brain tissue will be noted. Similar changes in the content of the poly-PI components in brain tissue also were observed in hypoxic hypoxia: keeping the rats in a pressure chamber at a pressure of 180-200 mm Hg for 2 h also led to a small (by 8%) but significant decrease in the TPI content and a well-marked (by 50%) increase in the DPI content in the rats' brain. Changes of a similar nature in the DPI and TPI content in the brain tissue of rats exposed to these two factors (hypoxic hypoxia and OPI poisoning) cannot be taken as fortuitous. It is well known that disturbances of respiration occupy a leading place in the picture of OPI poisoning and are the direct cause of death [1]. Depending on the nature of the cholinesterase inhibitors used, the hypoxia in different animals may arise by different mechanisms (bronchospasm, action on the respiratory center, excitation of the chemoreceptors of the carotid body, paralysis of the respiratory muscles), but each of these mechanisms can cause the development of a varied degree of hypoxia. The changes in the DPI and TPI content found in the brain of the experimental rats may presumably reflect the state of hypoxia of the brain tissue developing in OPI poisoning. These changes (together with the well-known biochemical changes such as a decrease in the content of high-energy phosphates, the accumulation of inorganic phosphate and lactic acid, etc.), may be a characteristic metabolic response of the brain tissue to hypoxia.

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