PHOSPHOLIPIDS IN THE BRAIN AND SERUM OF RATS SUBJECTED TO EXPERIMENTAL MANGANESE TREATMENT

L. Halacheva

Phospholipids are an essential constituent part of the cells. Ethanolamine phosphatine (EP), sphingomyelin (SPM), and to a lesser degree lecithin (L) and serine phosphatide (SP) enter in the myelin composition (16). The metal complexes of phospholipids are mainly concentrated in the cytoplasmic membrane, in the mitochondrial and nuclear fraction. Lecithin (phosphatidylcholine) and phosphatidylethanolamine (70—80 per cent of the total amount of phospholipids) build up the basic mass in phospholipid membranes. The brain membranes contain in addition phosphatidylserine (18), phosphatidic acid and its polymer polyglycerophosphatide (PGP). Presumably the permeability of nerve membranes may undergo modification owing to th, ability of inositol-containing phospholipids (monophosphoinositide phose phatide — MPIP) to form stable complexes with Ca_2^+ through their monoesterified PO_4^{-3} groups (1, 9). The exceptionally important role played by magnesium phospholipids in accomplishing the numerous nerve cell transformations is well known (11). Manganese whose electron configuration outlines it as a good chelate-producer, may participate in complex compounds with the phospholipids (5), where through Ca_2^+ and Mg_2^+ replacement, the biological properties of the compounds will be altered. The latter opinion is confirmed by our studies since we were successful in establishing a reduction of the above ions' concentration in the brain and serum of rats under manganese effect. Pyridoxalphosphate (PLP) is a specific co-factor, promoting the activation of sphingomyelin synthesis in the brain (3, 17), whereas manganese exerts effect on the PLP content. We found a reduction of the latter in the brain, liver and blood of animals treated with manganese (5).

The above findings gave us sufficient reason to surmise the occurrence of derangements in fat metabolism, respectively, in the concentration of pho spholipids under the effect of manganese.

Material and Methods

The study was conducted on 80 fully mature male white rats, weighing 130 ± 15 g in the average, and distributed in groups as shown in Table 1.

Serum phospholipids were demonstrated using the tests of Hanry, and in 10 per cent homogenate — according to the method of McMurray (14) as modified by Smirnov and co-authors (2) and Karagezyan (10). The staining of dots was carried out with iodine vapours (15). Phospholipids in the brain were calculated as lipidic P in mcg/g fresh tissue, and in the serum — in mg% phosphatides, resp. P.

Table 1

Distribution of Animals and Doses Employed

Groups	Number of animals		Dose
	Experimental days		
	30 days	60 days	
Controls	20	20	150 mg/kg Mn^{2+}
Poisoned	20	20	per os 10.7% solution $MnCl_2 . 2H_2O$

Results and discussion

Serum and brain phospholipid concentrations are shown in Tables 2 and 3.

Table 2

Phosphatides (mg %) in the Serum, resp. Lipidic Phosphorus

Groups	Phosphatides						Lipidic phosphorus					
	30 days			60 days			30 days			60 days		
	x	Sx	p	x	Sx	p	x	Sx	p	x	Sx	p
Controls	76.18±3.01			101.71±2.89			4.28±0.15			4.84±0.25		
Poisoned	43.60±1.93	0.001		74.32±2.46	0.001		1.82±0.24	0.001		3.16±0.10	0.001	

Table 3

Phospholipids in the Brain (P mcg/g fresh tissue)

Phospholipids	30 days					60 days				
	controls		poisoned		p	controls		poisoned		p
	x	Sx	x	Sx		x	Sx	x	Sx	
x-phospholipids	60.34±1.24		44.15±1.23		0.001	62.15±1.20		32.46±1.15		0.001
y-phospholipids	35.72±1.25		23.11±1.36		0.001	39.21±1.17		18.32±1.13		0.001
MPIP	81.62±1.13		62.20±1.15		0.001	94.35±1.22		56.25±1.15		0.001
SPM	230.15±21.60		181.63±17.10		0.001	252.71±22.50		154.30±15.60		0.001
L	792.03±23.11		598.32±14.71		0.001	841.42±25.00		503.17±16.24		0.001
SP	168.11±5.17		126.81±2.05		0.01	192.15±4.12		131.13±2.11		0.001
EP	389.05±4.20		356.44±3.62		0.01	412.10±5.01		341.86±3.14		0.001
PGP	94.61±1.52		81.12±1.42		0.01	107.12±1.87		74.65±1.36		0.001
Total	1851.63		1450.67			2001.21		1312.21		

From Table 2 it can be seen that serum phosphatides in the control animals show a nearly 1.3 increase with aging. Under manganese effect an almost twofold decrease is observed within 30 days of introducing the noxa (53 per cent), and to a lesser degree within 60 days (with about 34 per cent).

In the brain and serum of the control animals a certain age related increase in phospholipid concentration is also noted (with 1.08 per cent). These results comply with the data reported by Rouser and Kritchevsky (19).

Under the effect of manganese a reduction of all phospholipids included in the study is recorded, a reduction which is in direct correlation (r=0.99) with the duration of the experiment. Within 30 days, the total lowering amounts to 21.65 per cent, and within 60 days — to 34.43 per cent. At 30 days, the fall in lecithin content is the greatest (with 24.46 per cent), next ranking MPIP (with 23.79 per cent), without taking into consideration x- and y-phospholipid reduction. The latter show the strongest decrease in either of the experimental periods. After 60-day long poisoning, ihe lowest level is displayed by L and MPIP (with about 40 per cent), followed by SPM (with 38.94 per cent).

The obtained results corroborate our hypothesis about manganese treatment effect on phospholipid metabolism. Apart from formation of manganochelates and reduced PLP concentration, the lowered phospholipid concentration may be also explained by the established decreased concentration of free amino acids in the brain, blood and liver of animals and humans under the effect of manganese (7, 8). The latter presumption is in accordance with the data submitted by Karagezyan et al (12) concerning the lowered content of phospholipids in the brain of fasting animals. There are reports by a number of authors (2, 4, 13) on the splitting of individual phospholipids in the brain in cases of protein deficiency.

Our results may be furthermore related to the substantial increase in the level of fatty acids, found in the animals treated with manganese (6). In the opinion of Karagezyan (10), these acids participate in the synthesis of phospholipids, blood phospholipids in particular, but we feet that it is more likely that such an increase is produced by lipid decomposition, and not by a regulatory mechanism. Moreover, the substantial lecithin decrease in the brain may be attributed to the manganese induced reduction of S-adenosylmethionine content (5), indispensable in one of its synthesis routes (21).

Hence, the results described above point to a phospholipid metabolism derangement in experimentally induced manganese intoxication. The study of phospholipid content in the serum of workers exposed to manganese effect is of interest, and it will be the subject of further researches along this line.

REFERENCES

1. A b o o d, L. J. I Meeting of the Int. Sic. Neurochem. Strasbourg. 1967, 2—12. — 2. A b o o d, L. J., A. G e i g e r. *Am. J. Physiol.*, 182, 1955, 557—561. — 3. B r a d y, R. O., J. V. F o r m i c a, G. J. K o v a l. *J. Biol. Chem.*, 273, 1958, 1072—1076. — 4. G e i g e r, A. *Physiol. Rev.*, 38, 1958, 17—22. — 5. H a l a c h e v a, L. Dissert., Sofia, 1971. — 6. H a l a c h e v a, L. First Scientific Session on Chemistry with Med. Profile, Varna, 1975, 129—134. — 7. H a l a c h e v a, L., V. B o y a d j i e v. Fifth Scientific Session M. F. Varna, 1973. — 8. H a l a c h e v a, L., P. N i k o l o v a, V. B o y a d z h i e v, VI Scientific Session M. F. Varna, 1974. — 9. H a w t h o r n e, J. N., M. K a i. *Int. Con. Biochem.*, Abst. 4, Tokyo, 1967, 747. — 10. K a r a g e z y a n, K. G. *Vopr. Biochem. Mozga,* AN Arm. SSR, Erevan, 2, 1966, 136—140. — 11. K a r a g e z y a n, K. G., P. M a n a s i a n. *Vopr. Bioch. Mozga,* AN Arm. SSR, Erevan, 6, 1970, 203—211.—12. K a r a g e z y a n, K. G., S. S. S a h a k i a n, L. T. M a k a r i a n, A. M. O v s e n i a n. *Vopr. Biochim. Mozga,* AN Arm. SSR, Erevan, 3, 1967, 199—208. — 13. K n a u f f, H. G., F. B ö c k. *J. Neurochem.*, 6, 1961, 171—177. — 14. M c M u r r y, U. C., L. R o g e r s. *Can. J. Biochem.*, 51, 1973, 9, 1262—1267. — 15. N a k h a s i, H. L., A. D. T o r o s, L. A. H o r r o k s. *Brain Res.*, 83, 1975, 176—179. — 16. N o r t o n, W. T., L A. A n t i l o. *J. Neurochem.*, 13, 1966, 213—215. — 17. P a n g, R. L., A. K i r s -

key. *J. Nutr.*, 104, 1974, I, 111—115. — 18. Pigareva, Z. D. *Biochim. Mozga*, 1972, Med., Moskva, 256—282. — 19. Rouser, G., G. Kritcheasky. Sphingolipids, Sphingolipidosis and Allied Disorders, ed. B. W. Volk a. S. M. Aronsson, 19, 1972, Pl. Press, N. Y., L., 103—126. — 20. Smirnov, A. A., E. V. Tchirkovskaya, K. G. Manukian. *Biochimia*, 26, 1961, 1027—1032. — 21. Zborowski, J., M. G. Sarzala. *Post. Biochem.*, 20, 1974, 1, 23—34.

ФОСФОЛИПИДЫ МОЗГА И СЫВОРОТКИ У КРЫС ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ВОЗДЕЙСТВИИ МАРГАНЦЕМ

Л. Халачева

РЕЗЮМЕ

Сообщается о понижении концентрации общих фосфолипидов в сыворотке крови у крыс, травленных 150 мг/кг веса Mn_2^+ через день в продолжении 60 дней. В более слабой степени наблюдалось уменьшение фосфолипидов в мозговой ткани, что находится в прямой корреляции (ч=0,99) с продолжительностью травления. Устанавливаются более низкие концентрации лецитина, монофосфоинозит-фосфатидов и сфингомиелина. Высказываются предположения для объяснения влияния на фосфолипидный обмен при воздействии марганцем.