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From the Department of Biochemistry, College of Veterinary Medicine, Helsinki, Finland.

ALTERATIONS OF RAT BRAIN LIPIDS IN METHYL MERCURY INTOXICATION

By

K. Salminen

SALMINEN, K.: *Alterations of rat brain lipids in methyl mercury intoxication.* Acta vet. scand. 1975, *16*, 76—83. — Lipid alterations due to experimental intoxication by methyl mercury were studied in rat brain. The methyl mercury was administered perorally as a hydroxide bound to bovine liver protein. The lipids were separated by thin layer chromatography and the fatty acids identified by gas-liquid chromatography. The lipids studied included free fatty acids, cholesterol and the main phosphatidylesters, phosphatidylcholine and phosphatidylethanolamine.

The methyl mercury treatment resulted in an increase in total brain lipids and palmitic acid (16:0) in the free fatty acid fraction. This increase is interpreted as the cellular response to compensate for the loss of membrane proteins known to take place in the brain due to methyl mercury intoxication.

brain lipids; methyl mercury; methyl mercury intoxication; gas-liquid chromatography.

Alkyl mercury is accumulated in the brain and the poisoning caused by it is characterized by nervous symptoms involving conspicuous histopathological changes in the central nervous system (*Hay et al.* 1963, *Miyakawa & Deshimaru* 1969). The biochemical mechanisms underlying the tissue damage are known to only a limited extent (*Yoshino et al.* 1966). Since the CNS exhibits a high content of lipids, which are of the utmost importance for the proper structure and functioning of the organism, it seemed appropriate to analyze the brain lipids of experimentally intoxicated rats in seeking for changes at the molecular level.

MATERIAL AND METHODS

Intoxication of the animals

Methyl mercury chloride (K & K Laboratories, Inc., Plainview, N.Y.) was dissolved in benzene and shaken overnight with an aqueous suspension of newly prepared well-washed Ag_2O to convert the methyl mercury to water-soluble methyl mercury hydroxide (MeHgOH). The MeHgOH was added to a bovine liver homogenate made in tap water (1:1 w/v) and incubated for 20 hrs. under constant shaking. One ml of the slurry containing approx. 1.8 mg MeHgOH was administered to adult male Wistar rats once a day by gastric intubation. The control animals received the identical liver homogenate without the MeHgOH. In addition the rats had access to standard laboratory feed and water ad libitum.

When one of the experimental rats was found dead, all the rats were sacrificed by decapitation; this took place on the 15th day of the experiment. The brain was immediately excised, weighed and stored at —20°C for treatment with lipid solvents.

Extraction of lipids and thin layer chromatography

The brains were homogenized individually in 30 ml of chloroform-methanol (2:1, by vol.) after which 10 ml of chloroform was added and the homogenization repeated. Another 10 ml of distilled water was added and the mixture re-homogenized. The mixture was centrifuged, and the lower phase collected and evaporated to dryness under reduced pressure. The dry lipids were dissolved in chloroform and applied to glass plates (20 cm × 20 cm) coated with 0.5 mm thick silica gel H (E. Merck, Darmstadt, Germany) as a slurry in water. The chromatogram was developed by a system of ascending solvents consisting of light petroleum (b.p. 60—80°C)-diethyl ether-acetic acid (80:20:2, by vol.). The lipid areas were detected by spraying the plates with 2,7-dichlorofluorescein and identified by comparison with the appropriate standards. The separated neutral lipids and phospholipids were eluted from gel scrapings essentially as described by *Skipski et al.* (1964). For phospholipid separation the chromatogram was developed first with chloroform-methanol-28 % aqueous ammonia (65:25:4, by vol.) and then with chloroform-acetone-methanol-acetic acid-water (8:6:2:2:1, by vol.). The phospholipids were located by exposing the plates to iodine vapour. The amount of the lipid was determined by the gravimetric method of quantification.

Cholesterol was determined by the method of *Pearson et al.* (1953).

Identification of fatty acids by gas-liquid chromatography

The residues of the lipids were dissolved in benzene-methanol-boronfluoride (5:10:1, by vol.) and converted into methyl esters as described by *Metcalfe & Schmitz* (1961). The methylation was carried out in 65°C for 30 min. under a nitrogen atmosphere. Aliquots of the methyl esters were analyzed by a Perkin-Elmer gas chromatograph,

model 800, equipped with a 2.2 m stainless steel column, 0.125 inch in internal diameter, packed with EGS on Chromosorb W 80/100 mesh. The results are expressed in terms of the percentual share of the major fatty acid methyl esters of the total area of all peaks.

RESULTS AND DISCUSSION

The total dose of MeHgOH administered to the experimental group was approx. 25 to 30 mg, which did not cause any distinct symptoms of methyl mercury poisoning. Thus intoxication was still in the clinically tacit stage, the most prominent symptom being loss of weight. The weight-loss observed in the control animals is probably due to the repeated gastric intubation and liver slurry, to which the pellet-fed animals were not accustomed.

Table 1. Analytical data on the experimental animals. Each value calculated as mean ± s, derived from at least nine animals.

	Weight at the onset (g)	Weight at the end (g)	Brain weight (%) of body weight
MeHgOH treated	342.5 ± 28.1	307.6 ± 18.4	0.595 ± 0.03
Controls	333.2 ± 20.5	315.1 ± 16.6	0.579 ± 0.05

For the isolation of the lipid classes studied, TLC and gravimetric methods were used to determine the quantity of the lipids. The emphasis was on the purity of the fractions and not on the quantitative yield. The concentrations of the lipid classes recovered and fatty acid distribution are in general accordance with other reports for the brain (*Rathbone* 1965, *Altrock & Debuch* 1968, *Cuzner & Davison* 1968, *Horrocks* 1968, *Svennerholm* 1968, *Galli et al.* 1970, *Pumphrey* 1969, *Baker & Thompson* 1972). Since in neutral lipid separation the neutral diacylglycerols are difficult to separate from cholesterol, the last-mentioned was determined chemically. The neutral acylglycerols in the brain constitute only 2—3 % of the total lipid content (*Biran et al.* 1964, *Sun & Horrocks* 1969, *Sun* 1970); their contribution was therefore ignored, and emphasis was directed to the separation of free fatty acids, cholesterol and the quantitatively major phospholipids, phosphatidylethanolamine and phosphatidylcholine.

Table 2. Concentration of various lipid classes in the brain of MeHgOH-treated and control rats.

Lipid classes	MeHgOH treated	Controls
(mg/g wet tissue)		
Lipid, total	155.3 ± 21.7*	110.2 ± 22.2
Phospholipid, total	32.8 ± 9.2	33.1 ± 11.5
(μmole/g brain)		
Free fatty acids	8.2 ± 4.8	7.8 ± 3.7
Cholesterol	21.4 ± 7.2	25.9 ± 3.4
Phosphatidylcholine	12.0 ± 1.1	11.4 ± 0.6
Phosphatidylethanolamine	17.6 ± 3.6	16.4 ± 1.7

* $P < 0.01$

The MeHgOH treatment resulted in a significant increase ($P < 0.01$) of the total brain lipid concentration in the experimental group, as seen in Table 2. Since the free fatty acids, cholesterol and total glycerophosphatides were not altered, and taking the low content of neutral acylglycerols into consideration, the increase seems to have taken place in the sphingolipid fraction of the brain.

When the fatty acid profile of the free fatty acids and the acyl group profiles of the phosphatidylesters studied in the experimental animals are compared to those of the controls, only one alteration, i.e. the increase of palmitic acid (16:0) in the free fatty acid fraction is observed, as shown in Table 3. This finding is of considerable importance, since fatty acid synthetase, the enzyme complex that catalyzes the synthesis of fatty acids, produces predominantly (85—90 %) palmitic acid (*Brady* 1960, *Volpe & Kishimoto* 1972, *Sun & Horrocks* 1973, *Volpe et al.* 1973). When [$1-^{14}C$] palmitic acid was fed to rats, brain palmitate had most of the radioactivity in the carboxyl carbon, showing also a direct uptake of the tracer (*Dhopeshwarkar & Mead* 1969, 1970). Thus there is evidence of a direct uptake of fatty acids and especially palmitic acid by the brain. Whether originating from a direct uptake from the blood or synthesized by fatty acid synthetase, palmitic acid seems to be the main long-chain fatty acid utilized by the brain. The observed increase in palmitic acid in the free fatty acid fraction obviously reflects accelerated synthesis and/or increased uptake in response to the methyl mercury

Table 3. Fatty acid composition of brain lipids studied as percentage of total methyl esters.

Fatty acids	Free fatty acids		Phosphatidylesters: phosphatidylcholine		Phosphatidylesters: phosphatidylethanolamine	
	MeHgOH treated	controls	MeHgOH treated	controls	MeHgOH treated	controls
10:0	—	—	tr.	tr.	0.6 ± 0.1	0.3 ± 0.2
12:0	0.6 ± 0.6	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.7 ± 0.6	0.3 ± 0.1
14:0	0.6 ± 0.6	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.8 ± 1.0	0.2 ± 0.1
15:0	—	—	—	—	4.9 ± 2.2	4.0 ± 1.2
16:0	19.7 ± 2.8*	16.4 ± 2.1	33.8 ± 4.7	31.5 ± 3.9	7.4 ± 2.1	6.0 ± 1.1
16:1	2.9 ± 0.9	3.0 ± 1.1	2.0 ± 0.6	2.2 ± 1.2	1.8 ± 0.6	1.3 ± 0.7
17:0	—	—	—	—	5.2 ± 2.9	6.5 ± 1.7
18:0	23.4 ± 2.5	24.1 ± 2.2	24.1 ± 3.5	25.9 ± 2.5	26.0 ± 5.5	26.2 ± 4.4
18:1	28.9 ± 2.4	30.4 ± 2.7	29.6 ± 1.7	28.7 ± 3.8	25.4 ± 2.6	25.9 ± 3.4
18:2 ω 6	2.6 ± 1.6	3.7 ± 2.9	0.7 ± 0.5	0.5 ± 0.3	0.3 ± 0.1	0.5 ± 0.2
18:3	1.8 ± 2.9	2.8 ± 2.9	1.6 ± 0.8	1.0 ± 0.7	5.8 ± 1.1	6.6 ± 1.1
20:4 ω 6	19.2 ± 2.6	18.8 ± 2.9	7.1 ± 3.9	9.6 ± 4.9	8.9 ± 2.6	8.8 ± 3.2
22:4 ω 6	—	—	tr.	tr.	3.0 ± 1.6	3.9 ± 1.8
22:6 ω 3	—	—	tr.	tr.	9.2 ± 5.6	9.4 ± 6.7

* $P < 0.02$
tr. = trace, account less than 0.2 %.

administration. The palmitic acid fraction tended to be higher also in the phosphatidylesters studied, although the differences were not statistically significant. This may be due to the slow turnover of the brain phospholipids.

The slight lipid alterations in the brain are in sharp contrast with the numerous lipid modifications observed in liver due to methyl mercury intoxication (*Salminen* 1975). This finding is in line with the considerable degree of resistance and chemical stability of the CNS. An electronmicroscope study of the brains of experimentally intoxicated rats revealed pathological changes in the granular cells of the cerebellum, and above all in the nuclear membrane, but not in the mitochondria even in an advanced stage of the poisoning (*Miyakawa & Deshimaru* 1969). The reason why organic mercury compounds damage only the granular cells is unknown. However, the observed cellular nuclear membrane damage (*Miyakawa & Deshimaru*) seems to be in agreement with the hypothesis of the response of cells to inhibition of RNA synthesis as suggested by *Farber* (1971). The depression of palmitic and oleic acid desaturation to correspond-

ing monoenes in liver caused by methyl mercury treatment was also considered to be due to the inhibition of RNA synthesis (*Salminen*). The increased lipid content and the parallel increase of palmitic acid synthesis and/or uptake in brain may be an indication of the cellular response to compensate for the loss of membrane proteins due to inhibition of RNA synthesis by accelerated lipid accumulation.

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SAMMANFATTNING

Lipidförändringar i råtthjärnan vid metylkvicksilverförgiftning.

Lipidförändringar förorsakade i råtthjärnan av en experimentell kvicksilverförgiftning blev undersökt. Metylkvicksilvret ingavs peroralt som hydroxid, bundet till leverprotein. Lipiderna isolerades med tunnskiktskromatografi och fettsyrorna identifierades med gaskromatografi. De undersökta fetterna omfattade fria fettsyror, kolesterol och de viktigaste fosfolipiderna, fosfatidylkolin och fosfatidyletanolamin.

Metylkvicksilverbehandlingen framkallade en ökning av totala mängden hjärnlipid och palmitinsyra (16:0) i fria fettsyrors fraktion. Ökningen anses vara den cellulära reaktionen för att kompensera förlust av membranprotein, som sker i hjärnan vid kvicksilverförgiftning.

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Reprints may be requested from: K. Salminen, State Veterinary Medical Institute, Box 368, 00101 Helsinki 10, Finland.