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CHANGES IN FATTY ACID ELONGATION IN DEVELOPING MOUSE BRAIN BY MERCURY - COMPARISON WITH OTHER METALS

(Mouse; mercury; brain; fatty acids)

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SUMMARY

Hg^{2+} dramatically altered microsomal synthesis of very long chain fatty acids specific for myelin in mouse brain, as measured by lengthening of behenyl-CoA by means of malonyl-CoA in vitro. This alteration was found with Cu^{2+}, but not with various other metal ions, showing that changes in fatty acid synthesis could be due to the alteration of sulphydryl groups.

INTRODUCTION

Mammalian organisms are highly susceptible to the toxic effects of several metals. Among such metals, mercury (Hg) is well known as an environmental poison. Several reports of methyl mercury (MeHg) intoxications of epidemic proportions have been connected to industrial waste [1] and to the use of fungicide-treated seeds as food [2]. Most publications report effects of organic Hg on the nervous system, but the effects of inorganic Hg have hardly been examined.

Although other organs may accumulate more organic Hg than the brain, the first and most severe symptoms of intoxication appear in the nervous system [3]. Chronic maternal exposure to MeHg during gestation is known to damage the foetal brain irreversibly [4]. Morphological changes in myelin and demyelination have been demonstrated in 'foetal Minamata disease' [5]. The mechanism of the toxic effects, particularly at molecular level, is not well understood: which brain enzyme systems

Abbreviations: MeHg, methyl mercury.

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are first affected by the poison, which biochemical changes are first induced and how these are related to functional disturbances, remains unclear.

Compared to other tissues, the nervous system is very rich in lipids, most of which are present in membranes. Fatty acid biosynthesis is a fundamental event during maturation of the brain, and alterations in the activities of enzymes involved in very long chain fatty acid biosynthesis could have a profound effect on the integrity and stability of the myelin membrane.

Recent studies have shown that MeHg inhibits galactolipid accumulation in myelin [6–8] and changes the fatty acid composition of myelin cerebrosides. The most significant change observed is a decrease in the ratio of hydroxy- fatty acids to non-substituted fatty acids in myelin [9]. In vitro, it enhances ganglioside GM_3 synthesis in culture [10]. We have shown that myelin very long chain fatty acids are synthesized in the microsomes via 3 different systems: a de novo mechanism synthesizing palmitic acid; a first elongating system producing stearic acid; and a second elongating system synthesizing very long chain fatty acids, either saturated or mono-unsaturated. These systems differ at the level of the respective enzymes, and are directly related to myelination [11]. Our studies were performed to determine the effect of Hg on the second elongating system, which provides very long chain fatty acids specific for myelin galactolipids. Hg^{2+} was compared with other metal ions to provide information about the specificity of inhibition, as MeHg toxicity has usually been associated with changes in the activity of certain enzymes containing active sulphydryl groups, such as the Na^+,K^+-ATPase [12]. However, while the interaction of MeHg with enzymes containing SH-groups was originally proposed [13], it has also been questioned [14]. Since most studies were performed using organic Hg, this work was undertaken to determine whether inorganic Hg also alters the metabolism of fatty acids.

MATERIALS AND METHODS

C57 black mice of indiscriminate sex were used. Microsomal preparation from 18-day-old mice has been previously described [15]. Behenyl-CoA was synthesized as previously described [16], purity was checked by thin-layer chromatography on silica gel plates visualised under ulraviolet light (350 nm); the eluting solvent was butanol:water:acetic acid (50:30:20, v/v/v).

The incubation was performed as previously described for the determination of behenyl-CoA elongation by means of malonyl-CoA [17]. The assay mixture contained 50 μM [1,3-^{14}C]malonyl-CoA (2 mCi/mmol); 500 μM NADPH; 0.015 mM stearyl-CoA; 0.08 M potassium phosphate buffer, pH 6.9; 0.32 M sucrose; 0.94% NaCl; 2 μg phosphotransacetylase; and approx. 1 mg protein. Various metal ions ($HgCl_2$, $CuCl_2$ etc.) were added to the medium. The final volume was 1.0 ml and the mixture was incubated at 37°C for 1 h. The reaction was stopped by the addition of 0.5 ml of 4.5 M methanolic KOH. The mixture was saponified for 15 min in a

boiling water bath and then acidified with 0.5 ml of 5.5 M HCl. This mixture was extracted twice with petroleum ether and then dried under nitrogen. After methylation of the residue, fatty acid methyl esters were identified by thin-layer and gas chromatography. Radioactivity was determined in an Intertechnique scintillation counter using PPO and POPOP in toluene. Proteins were determined according to Lowry et al. [18].

RESULTS AND DISCUSSION

4% of the malonyl-CoA added to the test tube was found in lignoceric acid; 13% of the behenyl-CoA was elongated, 90% of the used [1,3-^{14}C]malonyl-CoA acted as a C_2-donor to behenyl-CoA (data not shown). This is in good agreement with our previous paper [11]. Synthesized fatty acids were lignoceric acid and a small amount of cerotic acid. Elongation of endogenous fatty acids was inhibited by 75% by behenyl-CoA, and de novo synthesis was impeded by phosphotransacetylase [10]. Hg^{2+} inhibited the reactions (50% and 50 μM) and Cu^{2+} seems also to be a very potent inhibitor of behenyl-CoA elongation (50% at 100 μM (Fig. 1). Various ions were also assayed (Ca^{2+}, Sn^{2+}, Ni^{2+}, Mn^{2+}, Cu^{2+}, Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Bi^{3+}). Only Mg^{2+} induces significant inhibition above 5 mM (Fig. 1). Both Cu^{2+} and Hg^{2+} are potent inhibitors of the elongation of behenyl-CoA, suggesting that

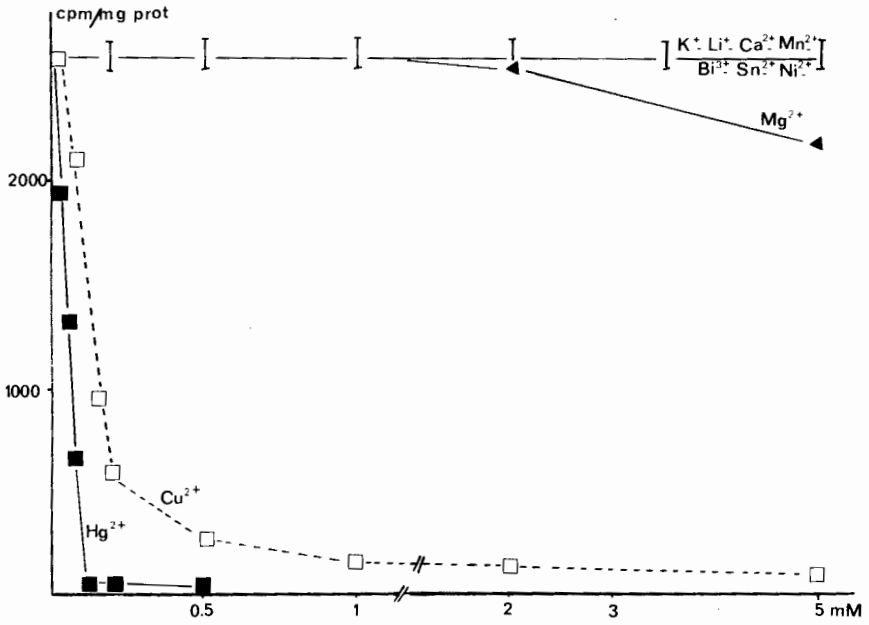

Fig. 1. Effect of mercury and various other metal ions on the elongation of behenyl-CoA. Abscissa: Metal concentration. Ordinate: [^{14}C]Malonyl-CoA radioactivity incorporated into fatty acids.

sulphydryl groups could be involved in the underlying reactions; but this is possibly contradicted by the fact that Sn^{2+} and Ni^{2+} are ineffective. Moreover, it has been shown that Hg^{2+} also inhibits non SH-enzymes [19]. Thus, impaired myelination during Hg intoxication could also be due to changes in the synthesis of very long chain fatty acids resulting from an alteration of enzyme activities at the level of sulphydryl groups.

This paper provides information about in vitro inhibition of fatty acid elongation by mercury; it is not possible to conclude that these results are of any significance in vivo.

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REFERENCES

1 L.T. Kurland, S.N. Faro and H. Siedler, Minamata disease. The outbreak of a neurological disorder in Minamata, Japan, and its relationship to the ingestion of seafood contaminated by mercuric compounds, World Neurol., 1 (1960) 370–391.
2 F. Bakir, S.F. Damhej, L. Amin-Zaki, M. Murtadha, A. Khalidi, N.Y. Al-Rawi, S. Tikuti, H.I. Dhahir, T.W. Clarkson, J.C. Smith and R.A. Doherty, Methyl-mercury poisoning in Iraq, Science, 181 (1973) 230–241.
3 K. Reuhl and L. Chang, Effects of methylmercury on the development of the nervous system: a review, Neurotoxicology, 1 (1979) 21–55.
4 D.H. Null, P.S. Gartside and E. Wei, Methyl-mercury accumulation in brains of pregnant, non-pregnant and fetal rats, Life Sci., 12 (1973) 65–72.
5 H. Matsumoto, G. Koya and T. Takeuchi, Fetal Minamata disease. A neuropathological study of two cases of intrauterine intoxication by methyl mercury compound, J. Neuropath. Exp. Neurol., 24 (1965) 563–574.
6 I. Grundt, H. Offner, G. Konat and J. Clausen, The effect of methyl-mercury chloride and triethyllead chloride on sulphate incorporation into sulphatides of rat cerebellum slices during myelination, Environ. Physiol. Biochem., 4 (1974) 166–171.
7 I.K. Grundt and N.M. Neskovic, Comparison of the inhibition by methylmercury and triethyllead of galactolipid accumulation in rat brain, Environ. Res., 23 (1980) 282–291.
8 I.K. Grundt, F. Roux, I. Treich, C. Loriette, J. Raulin and E. Fournier, Effects of methylmercury and triethyllead on Na^+K^+ ATPase and pyruvate dehydrogenase activities in glioma C_6 cells, Acta Pharmacol. Toxicol., 51 (1982) 6–11.
9 I.K. Grundt, E. Stensland and T.L.M. Syversen, Changes in fatty acid composition of myelin cerebrosides after treatment of the developing rat with methylmercury chloride and diethylmercury, J. Lipid Res., 21 (1980) 162–168.
10 G. Rebel, P. Guerin and K.N. Prasad, Effect of methylmercuric chloride on gangliosides of mouse neuroblastoma cells in culture, Lipids, 18 (1983) 664–667.
11 J.M. Bourre, Origin of aliphatic chains in brain, in N. Baumann (Ed.), Mutations Affecting Myelination, INSERM Symposium No. 14, Elsevier/North Holland, Amsterdam, 1980, pp. 187–206.

12 R. Patzelt-Wenczler and W. Schoner, Evidence for two different reactive sulfhydryl groups in the ATP-binding sites of ($Na^+ + K^+$)-ATPase, Eur. J. Biochem., 114 (1981) 79–87.
13 P.E. Brubaker, G.W. Lucier and R. Klein, The effects of methylmercury on protein synthesis in rat liver, Biochem. Biophys. Res. Commun., 44 (1971) 1552–1558.
14 Y. Yoshino, T. Mozai and K. Nakao, Biochemical changes in the brain in rats poisoned with an alkylmercury compound, with special reference to the inhibition of protein synthesis in brain cortex slices, J. Neurochem., 13 (1966) 1223–1230.
15 J.M. Bourre, S. Pollet, G. Chaix, O. Daudu and N. Baumann, Etude 'in vitro' des acides gras synthétisés dans les microsomes de cerveau de souris normales et Quaking, Biochimie, 55 (1973) 1473–1479.
16 J.M. Bourre and O. Daudu, Stearyl-alcohol biosynthesis from stearyl-CoA in mouse brain microsomes in normal and dismyelinating mutants (Quaking and Jimpy), Neuroscience Lett., 7 (1978) 225–230.
17 J.M. Bourre, M. Paturneau-Jouas, O. Daudu and N. Baumann, Lignoceric acid biosynthesis in the developing brain. Activities of mitochondrial acetyl-CoA dependent synthesis and microsomal malonyl-CoA chain elongating system in relation to myelination. Comparison between normal mouse and dysmyelinating mutants (Quaking and Jimpy), Eur. J. Biochem., 72 (1977) 41–47.
18 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurement with folin–phenol reagent, J. Biol. Chem., 193 (1951) 265–275.
19 K. Waku and Y. Nakazawa, Toxic effects of several mercury compounds on SH- and non SH-enzymes, Toxicol. Lett., 4 (1979) 49–55.