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Short communication

In vivo and in vitro effects of methylmercury on the activities of aminoacyl-tRNA synthetases in rat brain*

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Abstract. The activities of six aminoacyl-tRNA synthetase species were determined using enzyme preparations partially purified from the brains of control and methylmercury (MeHg)-treated rats. The activities of Asp-, Leu- and Tyr-tRNA synthetases were significantly reduced in the brains of MeHg-intoxicated rats, whereas those of Lys- and Met-tRNA synthetases remained unchanged. In contrast, the activity of His-tRNA synthetase was significantly increased in the symptomatic phase of MeHg intoxication. The activities of these six aminoacyl-tRNA synthetases in the control brains were affected to different extents on the direct addition of MeHg to the assay system in vitro. No positive correlation was observed between the in vivo and in vitro effects of MeHg on the enzyme activities. These results indicate that the aminoacylation of tRNA is one of the actions of MeHg, which leads to inhibition of protein synthesis, and it is suggested that the syntheses of cellular proteins may be modified in different ways by MeHg, depending on their amino acid compositions.

Key words: Methylmercury – Aminoacyl tRNA synthetase – Brain – Protein synthesis – Rat

Introduction

The alteration in the protein synthetic activity in the nervous tissues of MeHg-treated animals before the appearance of overt signs of methylmercury poisoning supported the notion that impairment of protein synthesis in nervous tissues is closely related to the neurotoxicity of this agent (for reviews, see Omata and Sugano 1985; Miura and Imura 1987). Changes in intracellular structures associated with protein synthesis were also observed in nervous tissues during the development of methylmercury intoxication in animals (Jacobs et al. 1977; Yip and Chang 1981). Although the quantitative aspect of methylmercury action on protein synthesis (Omata et al. 1980; 1982) and the modification of protein species (Kawamata et al. 1987) were demonstrated in nervous tissues of rats, it is not known which step(s) of the protein synthetic pathway or reactions are affected by methylmercury. Furthermore, little information is available as to whether the impairment of nerve functions caused by methylmercury is due to perturbation of the synthesis of critical protein species. Cheung and Verity (1985) showed that methylmercury affected the protein synthetic activity through a decrease in the activity of phenylalanyl-tRNA synthetase in the brains of infant rats. The present investigation was undertaken to assess the effects of MeHg on the activities of six aminoacyl-tRNA synthetase species in the brains of adult rats as the first step for clarification of the site(s) and mode of action of methylmercury on the protein synthetic pathway and the protein species.

Materials and methods

Animal treatment. Female Wistar rats weighing 200–230 g were used throughout the experiment. The rats received daily subcutaneous injections of methylmercury chloride (10 mg/kg) for 7 consecutive days. The development of MeHg intoxication in the animals was similar to that described previously (Omata et al. 1978; 1982) and they were killed on day 14 or 15, at which time typical crossing of the hind limbs when held by the tail, and body weight loss were seen. Control rats were treated similarly, with injections of the vehicle (10 mM $NaHCO_3$-Na_2CO_3, pH 9.2) alone, and were killed 7 days after the last injection.

Preparation of the pH 5 enzyme fraction. The pH 5 fraction from rat brain was prepared essentially according to the method of Cheung and Verity (1985), with modifications including the addition of PMSF to the homogenizing medium, centrifugation of the post-mitochondrial supernatant in a Hitachi RPS-65T swinging bucket rotor at 127000 g for 90 min and centrifugation of the solubilized pH 5 fraction at 1000 *g* for 60 s. The final preparation was used immediately or stored at −70° C until use within 1 week, although no appreciable change in the enzyme activity was found after storage at −70° C for 30 days.

Preparation of tRNA from the liver. Unfractionated transfer RNA was prepared from rat liver according to Nishimura (1971), except that the preparation was initiated with the post-nuclear supernatant of the homogenate mixed with potassium polyvinylsulfate (3 μg/ml). The tRNA fraction was stored at −70° C until use.

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Assay of aminoacyl-tRNA synthetase activity. In preliminary experiments to determine the optimal conditions for the determination of aminoacyl-tRNA synthetase activities, each synthetase activity was measured with various amounts of amino acids and the enzyme fraction (5–70 μg), together with rat liver tRNA (0–8.5 μg) for 5–30 min. It was found that the reaction was linear for about 20 min with 400 μM amino acids and the following enzyme fraction/tRNA amounts for the respective aminoacyl-tRNA synthetases: 20 μg/7.0 μg for Asp; 20 μg/2.5 μg for Leu, Tyr, Lys and Met; 10 μg/2.5 μg for His. The incubation mixture (50 μl), prepared in an Eppendorf centrifuge tube (1.5 ml), contained 10 mM Tris-acetate (pH 7.4), 10 mM Mg-acetate, 100 mM KCl, 5 mM ATP, 2.5–7.0 μg rat liver tRNA, 0.2 μCi of a ^{3}H-labelled amino acid and the respective cold amino acid to make the final concentration 400 μM, and the pH 5 enzyme fraction (10–20 μg protein). The reaction, initiated by adding the pH 5 enzyme fraction, was carried out at 37° C for 10 min, after which the mixture was cooled on ice. The following procedures were performed according to Bollum (1968) with slight modifications. Two aliquots (15 μl, each) were taken from a cooled reaction mixture and then pipetted on to cellulose filter papers (Toyo, No. 2, 15 × 15 mm), which were dropped into cold 5% trichloroacetic acid. Each filter paper was then washed three times with 10% trichloroacetic acid, once with ethanol:ether (1:1, v/v) and once with ether, followed by standing at 70° C for 30 min. For washing in the Tyr-, Met-, His- and Asp-tRNA synthetase assays, the trichloroacetic acid solution contained 10–100 mM of the respective cold amino acids to reduce nonspecific absorption of the labelled amino acids. The dried filter paper was placed in a glass vial and the radioactivity was determined after the addition of 10 ml scintillation fluid (0.4% diphenyloxazole in toluene). When the effect of MeHg in vitro was being determined, 8–12 reaction tubes, for an aminoacyl-tRNA synthetase assay, containing the control pH 5 enzyme fraction and various amounts of MeHg chloride were processed as described above, and then the IC_{50} value for each enzyme was calculated from the inhibition curve. All media, glassware, and pipettes were autoclaved before use. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Statistical analysis. The two-tailed Student's *t*-test was used to estimate the statistical significance of the difference between the means; $p < 0.05$ was regarded as significant.

Results and discussion

Yield of the pH 5 enzyme fraction in control and MeHg-treated rats

Table 1 shows the yields of the pH 5 enzyme fractions and protein concentrations of the cytosol fraction obtained from the brains of control and MeHg-treated rats. No significant difference in the yield of the pH 5 enzyme fraction was observed between control and MeHg-treated rats on the basis of tissue weight. Moreover, the two groups of rats did not show any appreciable differences in the protein concentrations of the cytosol fractions, from which the pH 5 enzyme fractions were prepared. These results indicate that the protein amounts of cytosol proteins and the pH 5 enzyme did not changed as a whole in the brains of MeHg-treated rats, although changes in the protein compositions of these fractions have not been seen.

Table 1. Yields of the cytosol protein and pH 5 enzyme fractions from the brains of control and MeHg-treated rats

Treatment (no. of rats)	pH 5 enzyme (mg protein/g tissue)	Cytosol (mg protein/ml)
Control (6)	5.72 ± 0.52	3.54 ± 0.23
MeHg-treated (6)	5.86 ± 0.51	3.49 ± 0.15

The cytosol and pH5 fractions were prepared as described under Materials and methods. Each value represents the mean ± SD for six animals

Table 2. Effect of MeHg in vivo on aminoacyl-tRNA synthetase activities in the brains of rats

Amino acids	pH 5 enzyme activities		MeHg/Control %
	Control	MeHg-treated	
Asp	9.16 ± 1.14[a]	5.66 ± 0.97	62*
Leu	19.65 ± 3.36	15.53 ± 0.77	79**
Tyr	20.14 ± 0.89	18.05 ± 0.53	89**
Lys	45.13 ± 5.94	49.97 ± 7.03	110
Met	50.17 ± 7.54	45.27 ± 3.29	90
His	20.32 ± 2.35	29.95 ± 3.89	148*

[a] Each value (fmol aminoacylated/μg protein/10 min) represents the mean ± SD for six rats
* $p < 0.001$ ** $p < 0.05$ *** $p < 0.01$

Table 3. Inhibition of aminoacyl-tRNA synthetase activities by MeHg in vitro

Amino acids	IC_{50}[a] (MeHg/protein)
Asp	98
Leu	23
Tyr	90
Lys	97
Met	24
His	138

[a] IC_{50} denotes the ratio (nmol MeHg/mg protein of pH 5 fraction) in the assay system at which the enzyme activity was reduced to 50% of the control level

Changes in aminoacyl tRNA synthetase activities in the brain of MeHg-treated rats

The activities of six aminoacyl tRNA synthetase species are shown in Table 2. The control activities ranged from 9.16 (fmol/μg/10 min) for Asp to 50.17 for Met. Significant decreases in the activities were observed for Asp, Leu and Tyr. In contrast, His tRNA synthetase activity exhibited a significant, about 50%, increase. The enzyme activities for Lys and Met remained unchanged on MeHg treatment. The requirement of sulfhydryl groups is known for several aminoacyl-tRNA synthetase species (Mehler and Chakraburtty 1971; Murayama et al. 1975; Som and Hardesty 1975). In order to determine whether or not the effects

of MeHg observed in vivo (Table 2) are due to the direct action of MeHg accumulated in the tissue, we examined the effect of MeHg added directly to the reaction mixtures on the synthetase activities in control rats. The results shown in Table 3 indicate that the effect of MeHg in vitro varied from enzyme to enzyme, the IC_{50} values ranging from 24 nmol/mg protein for Met to 138 nmol/mg protein for His. We expressed the effect of MeHg in vitro as nmol Hg/mg protein of the pH 5 fraction at IC_{50} instead of the absolute concentration (μM) of MeHg in the reaction mixture according to the previous consideration on comparison of the in vivo effects of MeHg on enzyme activities with those in vitro (Omata and Sugano 1986), since (1) we used crude enzyme preparations and (2) the amount of the pH 5 enzyme fraction added to the reaction mixture for the optimal assay differed for the six amino acids, being in the range of 7–20 μg protein/assay. From the results in Tables 2 and 3, it is presumed that the effect of MeHg in vivo cannot be a direct one. Firstly, no appreciable correlation between the effect of MeHg in vivo and that in vitro was observed; the Met- and Leu-tRNA synthetases were very sensitive in vitro but only the Leu-tRNA synthetase was inhibited in vivo, and the Asp- and Lys-tRNA synthetases showed similar sensitivities to MeHg in vitro but the Asp-tRNA synthetase alone was inhibited in vivo on treatment with MeHg. Secondly, the IC_{50} values in Table 3 are much higher than the level of accumulation of mercury (1–1.5 nmol Hg/mg protein) found in the brains of rats with a dosing schedule identical to the present one (Omata et al. 1978; 1986).

The present results indicate that the aminoacylation of tRNA is modified on MeHg treatment in vivo for the four amino acids examined and it is suggested that this step could be one of the targets of the MeHg action in the protein synthetic machinery in brain cells. Since the activities of six enzyme species were affected differently by MeHg treatment in vivo, it seems reasonable to assume that the syntheses of individual protein species may be modified differently depending on their amino acid compositions. Cheung and Verity (1985) demonstrated that Phe-tRNA synthetase activity was inhibited to 78% of the control level at about 1 day after a single injection (10 mg/kg) of MeHg into young rats (10–20 days after birth), whereas no effect was observed on the elongation of peptide chains and the formation of the initiation complex was rather stimulated by MeHg. The present study demonstrated that the step of aminoacylation was also significantly affected in adult rats during the symptomatic phase of MeHg poisoning. Several kinds of complexes of different aminoacyl-tRNA synthetases and membrane-bound synthetases are known in eukaryotic cells (Dang and Dang 1986). It is possible that changes in the synthetase activities caused by MeHg detected in the present experiments are due to alterations in the synthetic pathways for the enzyme proteins and/or association or dissociation of the enzyme complexes, as well as changes in the intracellular distribution of these enzymes caused by MeHg. Experiments are in progress to examine the mode of action of MeHg on apparent changes in the activities of aminoacyl-tRNA synthetases in the rat brain.

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