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Effects of Methylmercury *In Vitro* on Methionine Synthase Activity in Various Rat Tissues

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Methionine synthase (EC2.1.1.13) is a vitamin B_{12}-dependent enzyme that catalyzes the methylation of homocysteine to produce methionine. This function has considerable biological significance because methionine plays a crucial role in a number of important metabolic pathways such as protein synthesis, neurotransmitter production and myelination (Bender 1984; Reynolds 1976; Hoffman 1984). Furthermore, folate metabolism is dependent upon methionine synthase activity because the methyl group donated to homocysteine comes from methyltetrahydrofolic acid. The resulting tetrahydrofolic acid is used in a number of important metabolic pathways (Bender 1984; Chanarin 1982). The functional significance of this enzyme is reflected in the consequences of vitamin B_{12}-deficiency or specific inhibition of methionine synthase by nitrous oxide. These studies document a wide variety of adverse effects which include polyneuropathies (Bender 1984; Dinn et al. 1980), behavioral alterations (Reynolds 1976), blood dyscrasias (Chanarin 1982; Nunn 1984, 1987)), and reproductive disorders (Nunn 1984, 1987). Considering the scope and gravity of these deficits, it is surprising that so little effort has been devoted to the study of the effects of environmental toxicants upon methionine synthase function. Nitrous oxide is the only agent that has received more than a cursory inspection in this regard (Chanarin 1982; Nunn 1984, 1987; Deacon et al. 1980).

The purpose of this study was to expand our knowledge of the influences of toxicants upon methionine synthase activity. The toxicant chosen for study was methylmercury. It is recognized that methylmercury, along with other mercurials, pose a significant health risk due to the prevalence of their use in commercial and industrial applications worldwide (Berlin 1986). A mercury compound was chosen as the test toxicant because methionine synthase is a sulfhydryl enzyme (Stadtman 1971), and mercurials are known to bind avidly to sulfhydryl groups which accounts for most of their toxicities (Berlin 1986; Webb 1966). In this study we looked at the effects of methylmercury in vitro on the activity of methionine synthase in extracts from various tissues of the rat.

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MATERIALS AND METHODS

Adult female Sprague-Dawley rats (180-210 gm) were the tissue sources for all experiments. Animals were decapitated and brain, liver, kidney and ovary samples were immediately flash-frozen in methanol/dry-ice. The tissues were stored at -70°C until they were homogenized in chilled 50 mM Na-K phosphate buffer, pH 7.4 (1:9 w/v) using a Polytron homogenizer. The crude homogenates were then centrifuged for one hour at 8000 X g at 5°C. The supernatant fractions were then flash-frozen and stored at -70°C until use in the enzyme assay.

For each experiment a supernatant sample derived from a specific tissue of a single rat was thawed and divided into five aliquots. To each aliquot was added enough phenylmethylsulfonyl fluoride (PMSF) in dimethylsulfoxide (DMSO) to constitute a 100 μM concentration to inhibit protease activity. Immediately after the addition of PMSF four of the aliquots received different concentrations of methylmercury-HCl (MM) and one received the same volume of MM-free vehicle (distilled water). All five tissue aliquots were then placed in a water bath (37°C) and every 20 minutes duplicate samples (0.1 mL each) were taken from each of the five treatment groups. The methionine synthase activity in each sample was determined in a manner similar to that previously described by Koblin et al. (1981). Each experiment was performed three times using tissue samples from a different animal each time. The only exception was in the case of ovary analysis where only two experiments were conducted.

The methionine synthase activity was expressed as nmoles methionine produced per mg protein per hour. Bio/Rad Protein Assay Kits were used to determine sample protein concentrations using bovine serum albumin as the standard. Enzyme activity values were changed to percentages by dividing the amount produced in MM-treated groups by the amount produced in the vehicle-treated control group. The percentages were then plotted against preincubation time.

RESULTS AND DISCUSSION

Figures 1 and 2 illustrates the effects of exposure time and methylmercury concentration on methionine synthase activity in brain, liver, ovary and kidney. The responses in all four tissues were both time-dependent and dose-dependent, although there appears to be a definite difference in the magnitude of the response of methionine synthase to methylmercury between liver and all other tissues. In the case of the liver samples there was never a statistically significant difference between control and treated samples, even at the 300 μM concentration of methylmercury (Student's t test). However, there appeared to be a strong trend toward inhibition in the two higher concentrations where the coefficients of correlation were 0.79 and 0.74 respectively.

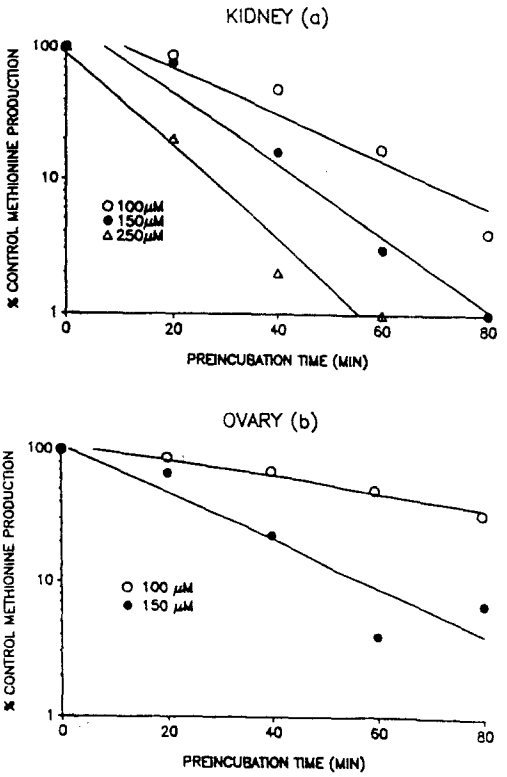

Figure 1(a-b). The effects of methylmercury concentration and preincubation time on methionine synthase activity in rat kidney and ovary.

Ovary and kidney methionine synthase appeared to be particularly sensitive to inhibition by methylmercury. In the ovary samples inhibition was complete after 40 min of preincubation in 250 μM methylmercury. When the concentration was raised to 300 μM, enzyme activity was essentially undetectable after only 20 min of preincubation. In kidney samples methionine synthase activity was only barely detectable after 20 min of preincubation in 300 μM methylmercury.

The information gained in the course of this project illustrates that low concentrations of methylmercury are capable of inhibiting the catalytic activity of methionine synthase in vitro. This finding was not altogether unexpected considering the large body of evidence detailing the inhibitory effects of mercurials on a wide variety of enzymes (Berlin 1986; Webb 1966). This ability is independent of the tissue source of the enzyme, although the enzyme activity in liver appears to be more resistant to methylmercury than that of brain, kidney and ovary.

The conclusion that methylmercury can inhibit methionine synthase in vitro corroborates and extends the results of a previous in vivo study (Brennt and Smith 1989). In this study, female rats

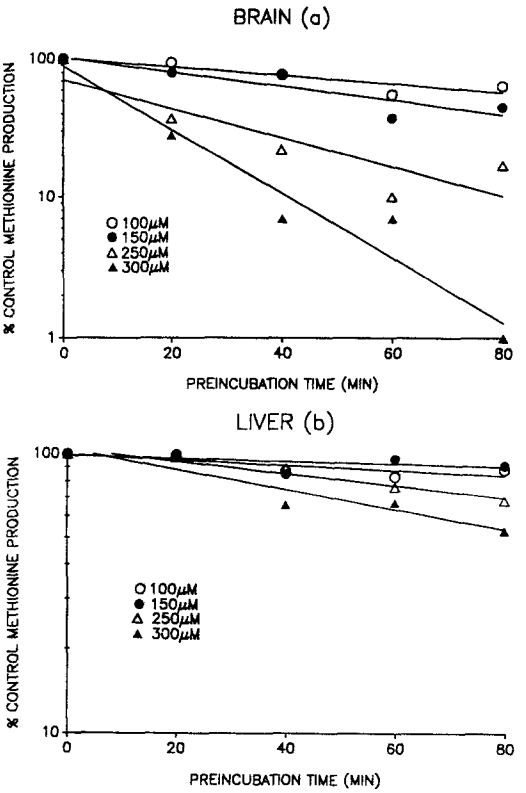

Figure 2(a-b). The effects of methylmercury concentration and preincubation time on methionine synthase activity in rat brain and liver.

were treated for four consecutive days with subcutaneous injections of saline or methylmercury (3 mg/kg or 6 mg/kg), and on the fifth day methionine synthase activity was determined in various tissues. Enzyme activities in the brain, spinal cord, and ovary were lowered in a dose-dependent manner while enzyme activity in the liver showed no definite trend.

In both of these studies there was an apparent insensitivity of methionine synthase in liver samples to the inhibitory action of methylmercury. The most parsimonious explanation for this observation would be that there is a substance, or substances, in the crude liver extract that binds methylmercury, thus lowering the actual concentration of free methylmercury available for interaction with methionine synthase. This hypothesis would be compatible with the observation that the liver is a major source of metallothionine-like substances and glutathione that are important metal chelating molecules in the body. Further purification of the crude extracts might help explain the diverse liver response noted in this study.

The potential toxicological significance of the inhibitory action of methylmercury on methionine synthase hinges on the question of

whether physiologically significant alterations of enzyme activity could be achieved at mercury levels attained under various exposure situations. In the large scale methylmercury intoxication in Iraq, blood levels of mercury in excess of 300 μg/100 mL were found (Hammond and Beliles 1980). Blood concentrations in the micromolar range were associated with significant frequency of paresthesias. It would seem reasonable to assume that methionine synthase was affected to some degree in these victims based upon the in vitro sensitivity we observed. Although the lowest concentration of methylmercury used in our study was 100 μM, there were dramatic decreases (40-80%) in methionine synthase activity after only one hour of exposure. This observation suggests that during the more prolonged exposures normally encountered in the occupational, or environmental, setting methylmercury might influence methionine synthase activity at much lower concentrations. In this regard it is worth noting that paresthesia, the early indicator of methylmercury toxicity in the Iraq exposure, is also an early indicator of toxicity resulting from chronic exposure to nitrous oxide, the potent inhibitor of methionine synthase (Brodsky et al. 1981).

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