EFFECTS OF METHYLMERCURIC CHLORIDE INTOXICATION ON THE INTRACELLULAR ACTIVITY OF LYPOSOMAL ENZYMES IN RAT LIVER AND BRAIN

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Received June 17, 1979

Abstract.....The activity of the lysosomal enzymes of rat liver after a single intraperitoneal injection of methylmercuric chloride (5.0 mg/kg) was enhanced in the nuclear fraction during early post-injection (2 hr to 7 days), while it decreased in the mitochondrial fraction and increased in the lysosomal fraction during late post-injection (14 days). Rat brain activity, however, was reduced in the nuclear, mitochondrial and lysosomal fractions during late post-injection (14 days). A high accumulation of total mercury was observed in the liver during early post-injection (36 hr and 7 days) while similar accumulation in the brain occurred during late post-injection (7 days and 14 days). Both the mercury burdens and the enzyme activities of the rat liver and brain returned to normal levels within 30 days.

Key words: methylmercury intoxication, acid phosphatase, β-N-acetylglucosaminidase, acid DNase, acid RNase.

INTRODUCTION

Organic mercury compounds have a marked affinity and toxicity to the organs, especially the kidney, liver and brain. It has also been recognized that the tissue distribution of mercury is in accord with the regions of pathological change in tissues (Berlin and Ullberg, 1963; Östlund, 1969; Miyakawa et al., 1969, 1970, 1973; Takah...
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Biochemical studies have shown that lysosomes containing a variety of acid hydrolases took up inorganic mercury, and that labilization of lysosomal membranes by mercury produced the release of lysosomal enzymes (Verity and Reith, 1967; Robinson et al., 1967; Norseth, 1968; Verity and Brown, 1970; Lauwers and Buchet, 1972; Mego and Barnes, 1973; Didelez et al., 1975). Furthermore, organic mercury compounds have also been shown to be stored in lysosomes of the liver, brain and kidney (Norseth and Brendeford, 1971; Miyakawa et al., 1973; Fowler et al., 1974), and the activities of acid phosphatase have increased slightly in these organs with increased mercury storage (Chang et al., 1973). The present investigation was designed to clarify correlative changes in the intracellular activity of the lysosomal enzymes and the total mercury burden of the liver and brain of rats during intoxication with methylmercury chloride.

MATERIALS AND METHODS

Male rats (Donryu strain, five rats per group) aged from 6 to 7 weeks were used. Intraperitoneally, the rats were given a single injection of 5.0 mg per kg methylmercuric chloride (Nakarai Chemicals, Kyoto) in saline. At a selected post-injection interval (2 hr, 36 hr, 7 days, 14 days and 30 days) animals were lightly anesthetized with ether. After bleeding, the tissues were perfused in situ with an ice-cold 0.25 M sucrose solution and removed. The tissue homogenates (15%, w/v) were prepared in an ice-cold 0.25 M sucrose-0.01 M Tris-HCl buffer, pH 7.4, in a Potter-Elvehjem homogenizer. Subcellular fractions were separated by centrifugation as described in Fig. 1. Four fractions, nuclear (N), mitochondrial (M), lysosomal (L) and microsomal-supernatant (M & S), from both the liver and brain homogenates were prepared. All precipitates obtained were suspended in the same amount of buffer as that used for homogenization and then Triton X-100 (Scintillation grade, Eastman Kodak) was added to a final concentration of 0.1%. The fractions were stored at -20°C until used for determination of enzyme activity. The whole blood was centrifuged at 3400 rpm for 20 min after standing at 4°C for 24 hr and the supernatant was prepared as a serum.

Acid phosphatase (EC 3.1.3.2), β-N-acetylg glucosaminidase (EC 3.2.1.30), acid DNase (EC 3.1.4.6) and acid RNase (EC 2.7.7.16) were assayed as described by Barrett (1972). Protein was determined by the method of Lowry et al. (1951) with crystalline bovine serum albumin as a standard. The substrates used for the enzyme assays (sodium β-glycerophosphate, E, Merk AG; p-nitrophenyl-β-N-acetylg glucosaminide, Calbiochem; calf thymus DNA, type V, Sigma Chemicals; and yeast RNA, sodium salt Kohjin Co. Ltd.) were commercial samples.

The total mercury of the tissue homogenate prepared above was determined when, after decomposition of the sample with a mixture of nitric acid and sulfuric acid, the mercury content in the aqueous phase obtained was measured by flameless atomic absorption spectrometry (Shoka et al., 1979).
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Rat (6-7 Week Age)

↓

5mg of CH₃HgCl/Kg

Blood Collection

Exanguination

Liver or Brain

Homogenization with 0.25 M Sucrose Buffer

Homogenate

↓

2,000 rpm for 10 min

Pellet (N Fraction)

Supernatant

↓

3,400 rpm for 20 min

Blood

↓

Serum

Pellet (M Fraction)

Supernatant

↓

13,500 rpm for 20 min (Liver) or 16,000 rpm for 30 min (Brain)

Pellet (L Fraction)

Supernatant (Ms & S Fraction)

Fig. 1. Centrifugation Scheme.

Fig. 2. Distribution of Total Mercury in Tissues of Rats After a Single Intraperitoneal Injection of Methylmercuric Chloride (5.0 mg/kg).
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RESULTS

Changes of mercury accumulation in tissues

Changes of total mercury content in the liver, brain, kidney and spleen of the rats during the course of methylmercuric chloride intoxication by a single intraperitoneal injection are presented in Fig. 2. The highest value in the liver was observed after 36 hr (4.78±1.31 μg/g wet weight) and that in the brain after 7 days (1.51±0.83 μg/g). However, the latter proved to be insignificant statistically (p<0.01). The highest concentrations in both the spleen and kidney were 16.75±8.11 μg/g and 22.03±2.97 μg/g, respectively, after 2 hr. The mercury burden in three of the tissues excluding the kidney, however, decreased to the normal levels of the untreated group at 30 days (treated: liver, 0.50±0.23 μg/g; brain, 0.86±0.36 μg/g; spleen, 1.01±1.08 μg/g; and kidney, 7.61±1.13 μg/g; and untreated: liver, 0.09±0.18 μg/g; brain, 0.67±0.85 μg/g; spleen, 0.73±0.39 μg/g; and kidney, 1.04±0.79 μg/g).

Changes with time in the enzyme activity in rat liver

Figure 3 shows the treated/untreated ratios of lysosomal enzyme activity and protein of the rat liver during the period of 2 hr to 30 days after methylmercuric chloride injection. Total activity of the four enzymes in the homogenate did not change significantly (p<0.01) with time. However, the specific activities of three enzymes, β-N-acetylglucosaminidase, acid DNase and acid RNase of the homogenate showed a decrease and the amount of protein a significant increase at 14 days. The ratio patterns of three of the enzymes, acid phosphatase, β-N-acetylglucosaminidase and acid DNase excluding acid RNase in the intracellular fractions were very similar to each other. A significant increase in the total activity of acid DNase and acid RNase was observed in the mitochondrial fraction at 2 hr, in β-N-acetylglucosaminidase at 36 hr, and in acid phosphatase, acid DNase and acid RNase at 7 days in the nuclear fraction. At 14 days, the total activities of three enzymes, acid phosphatase, β-N-acetylglucosaminidase and acid DNase, increased in the lysosomal fraction, but the specific activities of all four enzymes decreased significantly in the mitochondrial fraction. The specific activity of acid RNase showed a significant decrease in the lysosomal fraction at 36 hr and in the microsomal-supernatant fraction at 14 days. The amount of protein increased in both the lysosomal and microsomal-supernatant fractions at 14 days. Finally, at 30 days the total and specific activities of all four lysosomal enzymes and the amount of protein returned to the normal values of the control animals.

Changes with time in the enzyme activity in rat brain

Figure 4 shows the treated/untreated ratios of the lysosomal enzyme activities and protein of the rat brain during the course of methylmercuric chloride intoxication. The total activity of the four enzymes of the homogenate did not change with time, but the specific activities of acid phosphatase, β-N-acetylglucosaminidase and acid RNase decreased significantly (p<0.01) in the homogenate at 14 days. The ratio patterns of
Fig. 3. Activity Ratios of Four Acid Hydrolases in Each Fraction of Rat-Liver After a Single Intraperitoneal Injection of Methylmercuric Chloride (5.0 mg/kg). Data were expressed as a ratio value (treated rats/untreated rats) of the total activity (stars) and the specific activity (boxes) on a logarithmic scale; figures were significant ratio values (p<0.01) of the total activity (⋆) and the specific activity (★) and mean ± S.E. of five rats of each group; and fractions were homogenate (H), nuclear (N), mitochondrial (M), lysosomal (L) and microsomal-supernatant (Ms & S) fractions.

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Fig. 4. Activity Ratios of Four Acid Hydrolases in Each Fraction of Rat-Brain After a Single Intraperitoneal Injection of Methylmercuric Chloride (5.0 mg/kg). The details are the same as in Fig. 3.
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the four lysosomal enzymes were similar. Until 2 hr and 36 hr after injection no obvious changes were observed in the total and specific activities, respectively, of the four enzymes in all intracellular fractions. The total activities of β-N-acetylg glucosaminidase and acid DNase decreased first in the mitochondrial fraction at 7 days. At 14 days, the total and specific activities of all four enzymes decreased markedly in the nuclear fraction, the specific activities of β-N-acetylg glucosaminidase and acid RNase decreased in the mitochondrial fraction, and those of acid phosphatase and acid DNase decreased in the lysosomal fraction. The amount of protein showed a significant decrease in the nuclear fraction and a significant increase in the lysosomal fraction. Finally, the total and specific activities of the four lysosomal enzymes and the amount of protein returned to normal levels at 30 days as they did in the liver.

Changes with time in the enzyme activity in rat serum

The specific activities of the four lysosomal enzymes in rat serum were determined with a single intraperitoneal injection of methylmercuric chloride. No differences between the treated and untreated animals were, however, observed during the course of the 2 hr to 30 days after treatment (activities of untreated rat serum: acid phosphatase, 1.33 ± 0.43 μg/hr/mg of protein; β-N-acetylg glucosaminidase, 0.026 ± 0.010 μmole/hr/mg; acid DNase, 1.36 ± 0.31 μg/hr/mg; and acid RNase, 18.99 ± 1.62 μg/hr/mg).

DISCUSSION

A comparison of the results in Fig. 2 shows that, the mercury accumulation in rat liver reached its highest level at from 36 hr to 7 days (early post-injection) and that that of the brain reached its highest level at from 7 days to 14 days (late post-injection) after injection of a relatively low dose (5.0 mg/kg) of methylmercuric chloride. Their mercury burdens were, however, reduced to normal levels at 30 days. On the other hand, the activity changes of lysosomal enzymes of the liver were observed to increase mainly in the nuclear and mitochondrial fractions during early post-injection (2 hr to 7 days), while a decrease in the mitochondrial fraction and an increase in the lysosomal fraction took place during the late post-injection period (14 days). Those of the brain showed a decrease mainly in the three fractions, nuclear, mitochondrial and lysosomal, during the late post-injection period (14 days). The enzyme activities of the liver and brain returned to the normal levels at 30 days (Fig. 3 and 4). Thus changes in both the mercury accumulation and lysosomal enzyme activities were closely parallel in time. The results suggest that cell damage induced within tissues by methylmercury intoxication may occur first in the liver followed by the brain at the late period and is restored to normal within at least 30 days.

Brubaker and co-workers (1971, 1973) have reported that the synthesis of RNA and protein was enhanced in the liver and brain as the tissue mercury burden increased, while inhibitory effects were predominant in the kidney. They suggested that disturbances in protein synthesis are a fundamental aspect of methylmercury poisoning. In
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our results, the amount of protein increased in the lysosomal and microsomal-supernatant fractions of the liver at 14 days, these increases being due to the induction of new protein with methylmercury intoxication since the amount of protein increased in the homogenate per gram of the liver was increased. A high protein increase was also observed in the lysosomal fraction of the brain at 14 days although no change occurred in the homogenate. Present data indicate that the liver protein increase may have resulted from new production and accumulation of methylmercury-containing lysosomes during the convalescent period after cell damage as has been described in histochemical studies of the kidney (Fowler et al., 1974), liver (Ware et al., 1974) and brain (Miyakawa and Deshimaru, 1969; Miyakawa et al., 1970). Such new lysosomes may have served as mercury detoxicants (Norseth and Brendeford, 1971; Fowler et al., 1974).

Neither increase nor decrease in the activity of the four lysosomal enzymes of the serum was observed in our experiments. It is, therefore, probable that the releases of lysosomal enzymes in tissues into serum did not occur during the course of methylmercury intoxication.

SUMMARY

The correlative changes in the intracellular activity of four lysosomal enzymes, acid phosphatase, $\beta$-N-acetylglucosaminidase, acid DNase and acid RNase and the mercury burden of the liver and brain of rats were studied during the 2 hr to 30 days after a single intraperitoneal injection of methylmercury chloride (5.0 mg/kg). The mercury accumulation in the liver reached its highest level at 36 hr to 7 days while similar accumulation occurred in the brain at 7 days and 14 days. These mercury burdens were, however, reduced to normal levels at 30 days. The activity changes of lysosomal enzymes in the liver were observed at 2 hr to 7 days and 14 days, and those in the brain at 14 days after injection. The enzyme activities in the liver and brain returned to normal levels at 30 days. Thus changes in both mercury accumulation and lysosomal enzyme activity were closely parallel in time. The results suggest that the cell damage induced within tissues by methylmercury intoxication may occur first in the liver followed by the brain at the late period and is restored to normal within at least 30 days.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Prof. Dr. T. Ohno, Department of Pharmaceutical Analysis, Gifu College of Pharmacy, and his collaborators for the analysis of mercury by flameless atomic absorption spectrometry. This study was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

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