응용약물학회지, The Journal of Applied Pharmacology 6, 20-24(1998)

Thiamine Effects on Electroshock Seizure Threshold of Lead-exposed Rats

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(Received December 30, 1997; accepted March 10, 1998)

Abstract – In the present study, we tested whether lead intoxication induces change of the thiamine content and the seizure threshold in rats and the changes of seizure threshold are related to the changes of thiamine status. It was also tested whether administration of excessive thiamine could reverse the toxic manifestation of lead in rats. Four groups of Wistar rats were prepared: 1) control group, 2) lead treated group, 3) lead plus thiamine treated group, and 4) thiamine deficient group. Each group of animals was divided into three subgroups based on age: 3, 7 and 16 weeks. In each group, thresholds of electroshock seizure and thiamine contents in brain regions including telencephalon, brain stem, cerebellum were measured. Thiamine contents in brain regions of the lead treated group were significantly lower than those of the control group and thiamine treatment reversed the decrease back to the control level. Thresholds of the electroshock seizure of the lead treated group in 3, 7 week old rats and those of thiamine deficient group in 3 week old rats were significantly lower than those of the control group. These observations were reversed by the supplementation with thiamine. These results from the present study suggest that increased seizure sensitivity induced by lead intoxication in rats may be mediated at least in part through the changes of thiamine status.

Keywords □ lead toxicity, thiamine, rat brain, seizure sensitivity

The central nervous system is the most sensitive target of lead intoxication. Encephalopathy characterized with demyelination, abnormal conduction velocity, decrement of I. Q. score, seizures and coma are among the most serious complications of lead poisoning. Seizures are the most severe signs of lead poisoning. Alterations of seizure threshold and seizure responsiveness in lead exposed rats have been reported (Silbergeld *et al.*, 1979). Significant reductions of the doses required to produce seizures were found in lead-exposed rats for the convulsant drugs such as picrotoxin and strychnine. The intensity of maximal electroshock seizures was increased in lead-exposed rats (Silbergeld and Hruska, 1980). However the mechanism of lead neurotoxicity such as seizure and demyelination was not clear.

Lead toxicity has been suspected to be mediated through interactions between lead and endogenous substances that have high affinity to lead. Several researchers focused on the molecules that containing -SH group (Needleman and Bellinger, 1991). It was also reported that thiamine, an endogenous-SH containing molecule, can reduce the absorption of lead in gastrointestinal tract and enhance the elimination of lead from soft tissues including brain, kidney and liver (Ghazaly, 1991). Neurological defects such as peripheral neuritis and encephalopathy that occur frequently in lead intoxicated animals, have been observed in thiamine deficient animals, further supporting that the interactions between lead and thiamine exist to some extent (Keyser and De Bruijin, 1991).

In the present study, we investigated possible relationship between alterations of seizure sensitivity by lead intoxication and thiamine status. For this ends, we examined whether lead administration through drinking water influences the thiamine content in the brain of rats. And then, the changes of the seizure threshold induced by lead intoxication in rats may be related to the changes of thiamine status. It was also tested whether administration of excessive thiamine could reverse the toxic manifestation of lead in the lead intoxicated rats.

MATERIALS AND METHODS

Materials

Trichloroacetic acid, α-amylase, sodium hydroxide, thiamine hydrochloride, thiamine pyrophosphate were purchased from Sigma Chemical Co.(St. Louis, MO, U.S.

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A.). Lead acetate, ethylether, isobutanol, brom were purchased from Junsei Chemical Co.(Japan). Sodium bicarbonate(anhydrous), copper sulfate and sodium potassium tartarate were purchased from Yakuri Pure Chemicals Co., Ltd.(Japan). Thiamine tetrahydrofurfuryl disulfide (99.8%) was obtained from Il-Dong Pharmaceutical Company (Korea). Thiamine deficient diet was purchased from Sunkyo Farm Co. (Japan).

Animals and treatment

Wistar rats were supplied from the Laboratory Animal Center of Seoul National University. Four groups of animals were prepared: 1) Control group, 2) Lead treated group, 3) Lead plus Thiamine treated group, and 4) Thiamine deficient group. The control group received a normal diet and tap water, and the lead treated group received a normal diet and deionized and distilled water containing 0.2% lead acetate. The lead plus thiamine treated group was fed on a thiamine sufficient diet containing 2 mg thiamine tetrahydrofurfuryl disulfide per 1 kg of rat chow *ad libitum*, and given deionized and distilled water containing 0.2% lead acetate. The thiamine deficient group was fed on a thiamine deficient diet and tap water. Until experimental animals became 3-week old, lead and/or thiamine were administered through milk by giving lead and/or thiamine to their dams. After weaning, lead and/or thiamine were administered directly to experimental animals through drinking water or diet as described above. Animals were sacrificed by decapitation when they became 3-, 7- and 16-week old. Brains were rapidly removed from animals and dissected into three regions; telencephalon, brain stem (diencephalon/midbrain and pons/medulla), and cerebellum by the method of Glowinski and Iverson (1966) just before experiments.

Quantitation of Thiamine Content

Thiamine content in the brain tissue was measured by the thiochrome method reported by Rindi and deGiuseppe (1961). Briefly, brain tissues were homogenized in ice-cold 5% TCA and centrifuged. After digestion of the supernatant with by α-amylase at 48-50℃ for 30 min, it was washed with water-saturated ethylether, cyanogen bromide. Ice-cold 15% sodium hydroxide was added and the resulting thiochrome was extracted by isobutanol. Thiamine content was estimated using a spectrofluorometer (FP-777, Jasco international Co. Ltd.) at 425 nm with excitation at 358 nm (Edwin, 1979) and was expressed as μg/g wet tissue.

Measurement of Electroshock seizure threshold

Seizure was evoked by constant current stimulator and the resulting seizure was determined by overt hindlimb extension. Individual animal were treated by electroshocks of 0.2-sec stimulus duration with 2 mA increments or decrements in current intensity to calculate electroshock seizure threshold (Browning *et al.*, 1990). CC_{50} was determined by Litchfield-Wilcoxon's method (Litchfield and Wilcoxon, 1949).

Statistical Analysis

Data were expressed as the mean±S.E.M. For statistical evaluation of data, ANOVA test and Newman-Keul's test were used. Differences were considered statistically significant when $p<0.05$ was obtained.

RESULTS

Quantitation of Thiamine Content

Thiamine content in each brain region was shown in Fig. 1. Thiamine contents in the lead treated group were significantly lower than those of the control group and were about the same as those of the thiamine deficient group. In the lead plus thiamine treated group, thiamine contents were recovered to those of the control group.

Electroshock seizure threshold

Electroshock seizure threshold was shown in Fig. 2. Electroshock seizure thresholds of lead treated group in 3, 7 week old rats and thiamine deficient group in 3 week old rats were significantly lower than those of control group while thiamine treatment reversed the decrease back to the control level. Electroshock seizure thresholds of lead treated group in 16 week old rats and thiamine deficient group in 7 week old rats were lower than those of control group but the difference was not significant.

DISCUSSION

It has been reported that lead treatment through drinking water caused accumulation of lead in the brain of rats (Ryu *et al.*, 1995). Interestingly, supplementation of thiamine through diet significantly reduced the accumulation of lead in the brain, suggesting possible interactions between lead and thiamine. In addition, the thiamine deficiency by lead intoxication reduced the transketolase activity. That is thiamine-dependent.

In the present study we demonstrated that total thiamine contents are decreased in the brain of lead-intoxi-

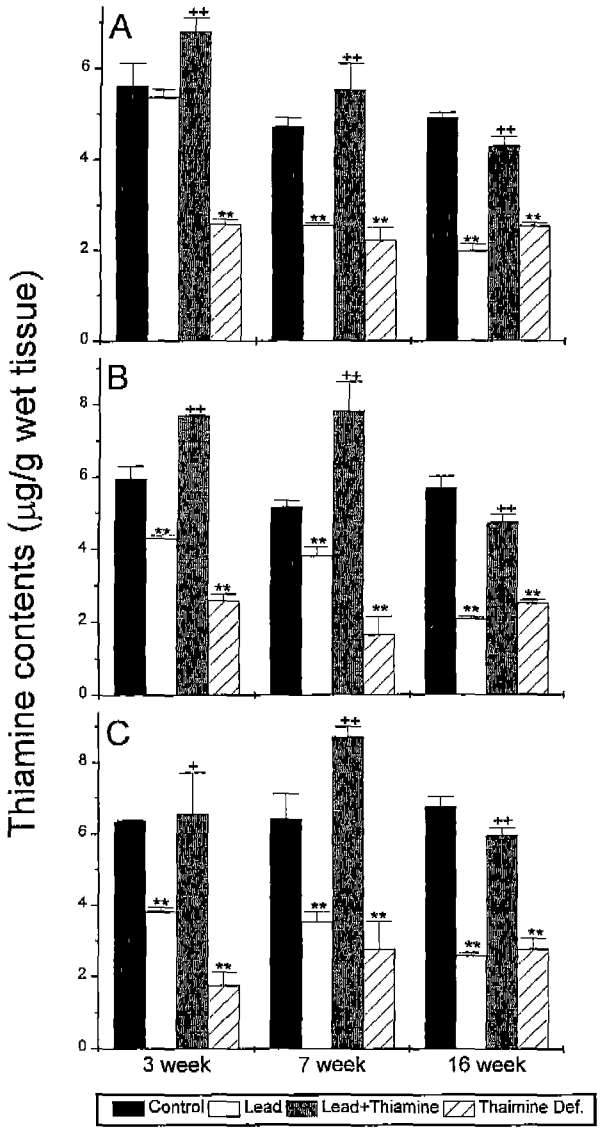

Fig. 1. Total thiamine contents in brain regions of 3, 7 and 16 week old rats. Each bar represents the mean±S.E.M. of data from 5 experiments. Panels A, B and C show the results obtained from telencephalon, brain stem and cerebellum, respectively. *indicates a significant difference from the control group (*, $p<0.05$ and **, $p<0.01$). +indicates a significant difference from the lead-treated group (+, $p<0.05$ and ++, $p<0.01$).

cated rats (Fig. 1). The decrement was more clearly shown as duration of lead treatment was increased. The reduced thiamine content in the brain may lead to pathological consequences since thiamine takes important roles in the central nervous system. Thiamine is an essential factor in brain energy metabolism and biosynthetic pathways (Gaitonde *et al.*, 1983; Gibson *et al.*, 1984; Parker *et al.*, 1984). In addition, thiamine is involved in the maintenance of normal membrane function

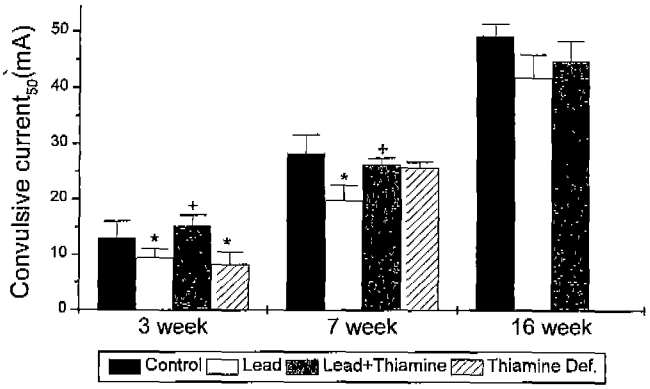

Fig. 2. Effects of lead intoxication on electroshock seizure in 3, 7 and 16 week old rats. Each bar represents CC_{50}+upper C. L. (95% confidence limits). *indicates a significant difference from control group ($p<0.05$). +indicates a significant difference from lead-treated group ($p<0.05$).

and the nerve conduction. Furthermore, many reports on the thiamine deficiency in experimental animal demonstrated the important roles of thiamine in the nervous system. Recently, the early morphological brain lesions in thiamine deficient rats are reported to be linked to energy deficiency. Thiamine depletion affects neurons and their functions in several specific areas of the central nervous system (Cooper and Pincus, 1979). Neurological defects, such as peripheral neuritis and encephalopathy, have been recognized in association with thiamine deficiency (Takashashi, 1981).

The decreased level of thiamine content in the brain of the lead treated group was recovered toward control level in the brain of the lead plus thiamine treated group (Fig. 1). This result may indicate the possible use of thiamine in the treatment of lead intoxication. First, administration of excessive thiamine reduces lead concentration in the body by blocking lead absorption as well as enhancing lead excretion. Second, it prevents deficiency of thiamine induced by lead intoxication.

Electroshock seizure threshold was decreased by lead intoxication (Fig. 2). Previous reports demonstrated that the intensity of maximal electroshock seizures was increased in lead exposed rats (Silbergeld and Hruska, 1980). The magnitude of decrement of electroshock seizure threshold induced by lead intoxication was nearly identical with that induced by thiamine deficiency. Electroshock seizure threshold of lead treated group in 3, 7 week old rats and thiamine deficient group in 3 week old rats were significantly lower than those of control group, while those of the lead plus thiamine treated

group were indistinguishable from those of control group. Decrement of electroshock seizure threshold induced by lead intoxication was observed in 3 and 7 week old animals with the concomitant decrement of thiamine content in all the brain regions tested. These decrements were reversed by the supplementation with thiamine. The results suggest that the changes of electroshock seizure threshold induced by lead intoxication may be related with changes of thiamine status, that is, lead intoxication can induce thiamine deficiency which may result in neurobehavioral changes such as increased seizure sensitivity.

Electroshock seizure threshold of lead treated group and thiamine deficient group in 16 week old rats were lower than those of control group but the differences were not significant in contrast with 3, 7 week old rats. It is possible that general adaptation mechanism to the same repetitive stimuli may cause this result. Adaptation mechanisms similar to this, have been reported in case of the other neuro-response (Winston *et al.*, 1990; Hope *et al.*, 1994). Factors which influence the development of electroshock seizure pattern include synaptogenesis, myelination, maturation of monoaminergic systems, and electrolyte and amino acid changes in rat brain (Fox, D. A. *et al.*, 1979). Demyelination and delays in myelin formation have been reported following lead intoxication and thiamine deficiency. The changes of electroshock seizure threshold induced by lead intoxication may be related with changes of myelin formation induced by thiamine deficiency or lead intoxication.

The results from the present study suggest that increased seizure sensitivity by lead intoxication in rats may be mediated at least in part through the changes of thiamine status.

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