Metallothionein Induction in Rat Brain after Intrastriatal Injection of Zinc and Cadmium Salts

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Brain parenchyma is protected against excess metals by the barrier system in the brain. To evaluate the expression of metallothionein (MT), a protective protein against heavy metals, in the brain parenchyma, zinc sulfate (0.2 or 2 μmol) or cadmium chloride (2 or 20 nmol) was injected into the left striatum of rats. Seventy-two h later, the MT level in the ipsilateral striatum injected with 0.2 μmol of zinc sulfate was not significantly higher than that after injection with vehicle. When the striatum was injected with 2 μmol of zinc sulfate, on the other hand, the MT level in the ipsilateral striatum, showing apparent degeneration, was significantly higher than that after injection with vehicle: the former was approximately 1.5 times the latter. In the case of injection with cadmium chloride at doses of 2 and 20 nmol, the MT level in the ipsilateral striatum was approximately twice that after injection with vehicle. The MT levels in the contralateral striatum and other brain regions were not affected by injection with either metal salt at any dose. When zinc sulfate of 100 μmol/kg body weight, corresponding to the lower dose tested (0.1 μmol/g brain), was subcutaneously injected, the hepatic MT level was approximately four times higher than the normal hepatic level. These results suggest that the changes of MT level in the brain were small compared to those observed in the liver.

Key words —— zinc, cadmium, brain, metallothionein

INTRODUCTION

There are blood—brain and blood—cerebrospinal fluid (CSF) barriers in the brain. Brain autoradiography with ^{65}Zn or ^{54}Mn demonstrated that zinc and manganese are transported into the brain *via* the blood—CSF barrier as well as the blood—brain barrier.[1−3] These metals are necessary for the growth and function of brain.[4−7] On the other hand, brain autoradiography with ^{109}Cd demonstrated that this metal, which is known to be toxic and may not be necessary for the brain growth and function, is hardly transported into the brain.[8] Therefore, the brain barrier systems are important for regulation of the uptake of metal ions into the brain and for maintenance of homeostasis in the brain. Excessive metals, even essential trace metals, are toxic to the brain. On the other hand, although the brain parenchyma is protected by the barrier systems, it may be susceptible to injury or degeneration by many materials including heavy metals.

In addition to the case of destruction of the blood—brain and blood—CSF barriers in association with neurological disorders, absorption of metals from the olfactory epithelium can circumvent the blood—brain and blood—CSF barriers in the process of transport into the brain and may lead to excessive accumulation of metals in the brain. Clinical studies on workers chronically exposed to heavy metals, *e.g.*, cadmium, demonstrated that such exposure may result in olfactory dysfunction.[9,10]

Metallothionein (MT) is an intracellular Zn-binding protein and is involved in detoxification of heavy metals.[11] MT genes are expressed in most tissues of almost all organisms and are regulated by multiple factors including metals, glucocorticoids and cytokines. MT-I and -II are expressed in the brain, primarily in ependymal and glial cells.[12,13] MT-III, a brain-specific isoform, was later discovered by Uchida *et al.*[14] In the present study, MT levels in rat brain after intrastriatal injection of zinc and cadmium were measured to evaluate the protective mechanism

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against the toxicity of these metals.

MATERIALS AND METHODS

Chemicals —— ^{203}Hg-mercuric chloride ($^{203}HgCl_2$) in diluted HCl (pH 1—2) was obtained from Amersham International plc.

Determination of MT —— The level of tissue MT was determined by the ^{203}Hg-binding assay of Piotrowski *et al.*[15] with a minor modification.[16] One ml of tissue homogenate (3 % w/v) in 0.1 M Tris-HCl buffer (pH 7.6) was incubated with 5 μl of diethylmaleate at 25 ℃ for 15 min. To the mixture was added 25 μl of 10 mM $CdCl_2$, and the mixture was then heated at 100℃ for 3 min to denature high-molecular weight proteins. To the supernatant obtained by centrifugation of the mixture at 600 × *g* for 10 min was added $^{203}HgCl_2$ solution (3.7 kBq/50 nmol, 50 μl). Then, after addition of 500 μl of 1 mM bovine serum albumin to remove non-MT-bound ^{203}Hg, 100 μl of 50 % trichloroacetic acid solution was added, and the mixture was centrifuged at 600 × *g* for 10 min. Radioactivity of the supernatant was measured in a gamma counter (Packard 5530).

Intrastriatal Injection of Metal Salts —— Male rats of the Wistar strain (130—150 g) (Japan SLC Inc.) were anesthetized with pentobarbital and individually placed in a stereotaxic apparatus. Through a small hole drilled in the skull was injected 10 μl of 20 or 200 mM zinc sulfate, 0.2 or 2 mM cadmium chloride in Ringer's solution or vehicle at the rate of 0.5 μl/min into the left striatum *via* a microdialysis probe without dialyzing membrane by using a microinjection pump (CMA/100, CMA Microdialysis) at the coordinates of 0.2 mm anterior to the bregma, 3.2 mm lateral to the midline suture and 5.5 mm interior from the dura.

RESULTS AND DISCUSSION

Several studies have indicated that MT expression in the brain is relatively unperturbed by systemic administration of zinc or cadmium at doses that strongly induce its expression in the periphery.[12,17,18] The small induction of MT in the brain may be due to the protective role of the blood—brain and blood—CSF barriers.

On the other hand, a significant induction of MT was observed in the case of intracerebroventricular injection of zinc sulfate.[12,19] However, the transport of zinc from the CSF was very slow in a tracer experiment using $^{65}ZnCl_2$ and a large portion of ^{65}Zn was retained in the CSF 24 h after the intracerebroventricular injection.[2] The ependymal or pial surface may regulate the transport of zinc from the CSF. It is thought that MT expression in the brain after intracerebroventricular injection of zinc sulfate may be induced by multiple factors. In the case of intracerebroventricular injection of $^{109}CdCl_2$, most ^{109}Cd was detected in the CSF 6 d after the injection and the ^{109}Cd level in the brain parenchyma was below the detection limit.[8] Therefore, it may be worth while to determine MT levels

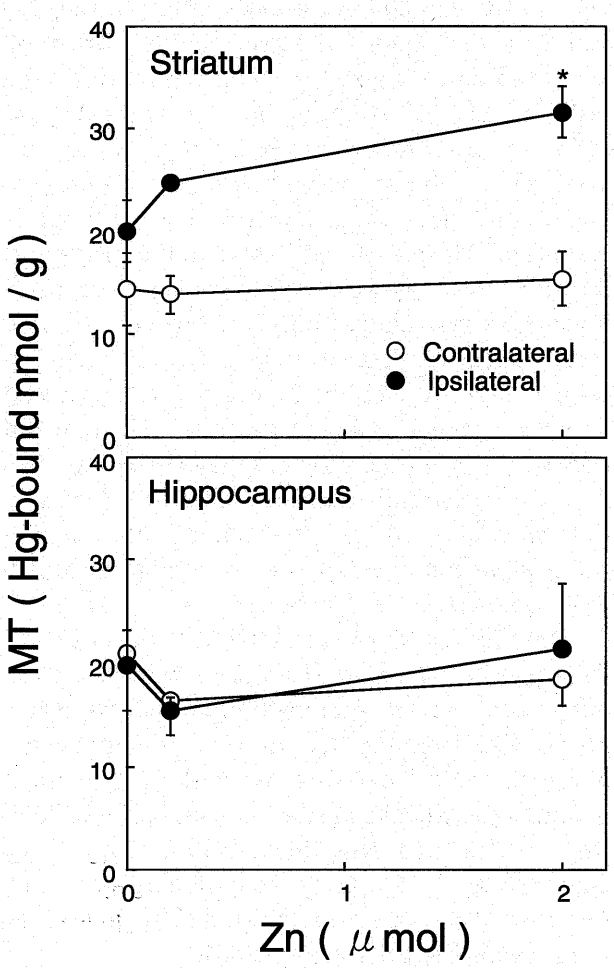

Fig. 1. MT Levels after Intrastriatal Injection of Zinc Sulfate

Ten μl of 20 or 200 mM zinc sulfate in Ringer's solution or vehicle was injected into the left striatum of rats. After 72 h, the MT levels were determined by the ^{203}Hg-binding assay. Each point and bar indicate the mean±S.D. ($n=4$), respectively. The asterisk indicates a significant difference (*, $p<0.001$) from vehicle control. There was no significant difference in the MT levels for the cerebral cortex, thalamus, mesencepharon as well as in the hippocampus between the groups injected with zinc sulfate and vehicle.

after injection of these metals into the extracellular fluid in the brain.

The biological half-life of zinc in the brain was estimated to be 16—43 d.[4] Seventy-two h after intrastriatal injection of $^{65}ZnCl_2$, a large portion of ^{65}Zn in the brain was detected in the striatum.[20] In the present study, MT levels in the rat brain after unilateral injection of zinc sulfate (0.2 or 2 μmol) and cadmium chloride (2 or 20 nmol) into the striatum were measured 72 h after injection by the ^{203}Hg-binding assay.

The MT level in the ipsilateral striatum did not increase significantly after injection with 0.2 μmol of zinc sulfate (Fig. 1). In the case of injection with 2 μmol of zinc sulfate, on the other hand, the MT level in the ipsilateral striatum, which showed apparent degeneration, was significantly higher than that after injection with vehicle: the former was approximately 1.5 times the latter. The MT levels in the contralateral striatum and other brain regions were not affected by the injection with zinc sulfate or vehicle. In the case of injection with 2 nmol of cadmium chloride, the MT level in the ipsilateral striatum was approximately twice that after injection with vehicle (Fig. 2). However, the MT level in the ipsilateral striatum after injection with 20 nmol of cadmium chloride, which caused apparent degeneration in the injected area, did not increase appreciably compared to the injection with one-tenth the dose. The MT levels in the contralateral striatum and other brain regions were not affected by injection with cadmium chloride at either dose.

In the case of intrastriatal injection of $^{65}ZnCl_2$, ^{65}Zn transport from the striatum *via* the extracellular fluid was negligible and the transport of ^{65}Zn from the struatum was mainly *via* the nerve fibers.[20] Therefore, it is likely that most zinc accumulates in the neurons and glial cells in the injected area after intrastriatal injection of zinc sulfate. This may be an explanation for the fact that MT was hardly induced in brain regions other than the ipsilateral striatum.

When zinc sulfate of 100 μmol/kg body weight, corresponding to the above lower dose (0.1 μmol/g brain), was subcutaneously injected, the hepatic MT level was approximately four times higher than the normal hepatic level 24 h after injection (data not shown). These results suggest that the changes of MT level in the brain are small compared to those observed in the liver.

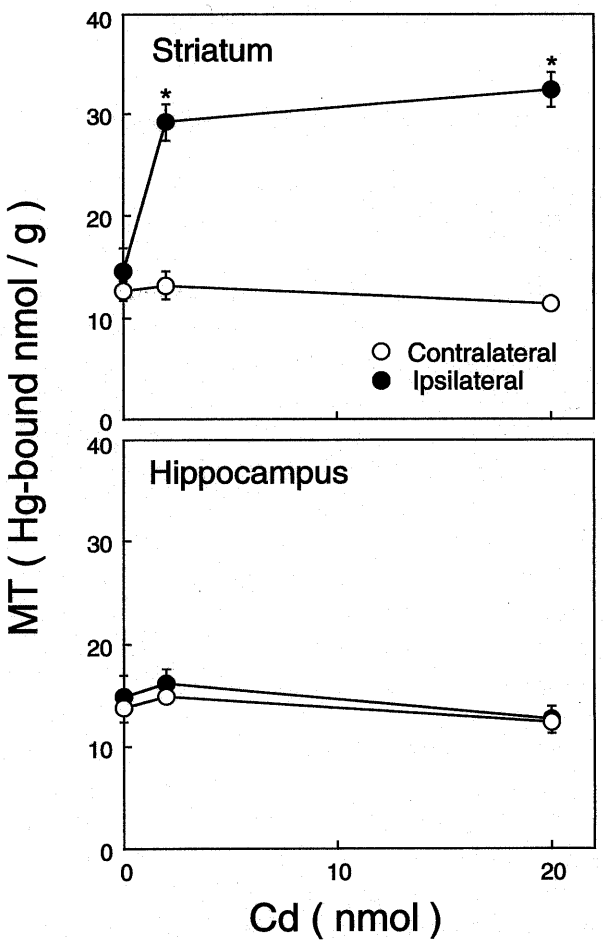

Fig. 2. MT Levels after Intrastriatal Injection of Cadmium Chloride

Ten μl of 0.2 or 2 mM cadmium chloride in Ringer's solution or vehicle was injected into the left striatum of rats. After 72 h, the MT levels were determined by the ^{203}Hg-binding assay. Each point and bar indicate the mean±S.D. ($n=4$), respectively. The asterisks indicate significant differences (*, $p<0.001$) from vehicle control. There was no significant difference in MT levels of the cerebral cortex, thalamus, mesencepharon as well as in the hippocampus between the groups injected with cadmium chloride and vehicle.

In the present study, the ^{203}Hg-binding assay was used to measure MT levels in the brain. Both levels of MT-I and -II are detectable by the ^{203}Hg-binding assay.[15] On the other hand, MT-III in addition to MT-I and -II is present in the brain.[14] Kramer *et al.* demonstrated that zinc and cadmium induce MT-I and -II mRNA but not MT-III mRNA in astrocytes and neurons *in vitro*,[21,22] suggesting that, in the case of intrastriatal injection of zinc or cadmium, at least MT-I and -II levels in the brain are measurable by the ^{203}Hg-binding assay. The small induction of MTs in the brain may lead to an increased susceptibility of neurons and glial cells to metal toxicity.

REFERENCES

1) Takeda A., Akiyama T., Sawashita J., Okada S., *Brain Res.*, **640**, 341—344 (1994).
2) Takeda A., Sawashita J., Okada S., *Brain Res.*, **658**, 252—254 (1994).
3) Takeda A., Kawai M., Okada S., *Brain Res.*, **769**, 193—195 (1997).
4) Takeda A., Sawashita J., Okada S., *Brain Res.*, **695**, 53—58 (1995).
5) Sawashita J., Takeda A., Okada S., *Dev. Brain Res.*, **102**, 295—298 (1997).
6) Takeda A., Kodama Y., Ishiwatari S., Okada S., *Brain Res. Bull.*, **45**, 149—152 (1998).
7) Takeda A., Ishiwatari S., Okada S., *Brain Res.*, **811**, 147—151 (1998).
8) Takeda A., Takefuta S., Ijiro H., Okada S., Oku N., submitted.
9) Friberg L., *Proc. Int. Congr. Ind. Med.*, **9**, 641—644 (1948).
10) Baader E.W., *Ind. Med. Surg.*, **21**, 427 (1952).
11) Suzuki K.T., Imura N., Kimura M., "Metallothionein III : Biological Roles and Medical Implications," Birkhauser, Basel, 1993.
12) Ebadi M., *Biol. Trace Elem. Res.*, **11**, 101—116 (1986).
13) Nishimura N., Nishimura H., Ghaffer A., Tohyama C., *J. Histochem. Cytochem.*, **40**, 309—315 (1992).
14) Uchida Y., Takio K., Titani K., Ihara Y., Tomonaga M., *Neuron*, **7**, 337—347 (1991).
15) Piotrowski J.K., Bolanowska W., Sapata A., *Acta Biochim. Pol.*, **20**, 207—215 (1973).
16) Takeda A., Sato T., Tamano H., Okada S., *Biochem. Biophys. Res. Commun.*, **189**, 645—649 (1992).
17) Choudhuri S., McKim J.M. Jr., Klaassen C.D., *Toxicol. Appl. Pharmacol.*, **119**, 1—10 (1993).
18) Zheng H., Berman N.E., Klaassen C.D., *Neurochem. Int.*, **27**, 43—58 (1995).
19) Gasull T., Giralt M., Hernandez J., Martinez P., Bremner I., Hidalgo J., *Am. J. Physiol.*, **266**, E760—E767 (1994).
20) Takeda A., Kodama Y., Ohnuma M., Okada S., *Brain Res. Bull.*, **47**, 103—106 (1998).
21) Kramer K.K., Liu J., Choudhuri S., Klaassen C.D., *Toxicol. Appl. Pharmacol.*, **136**, 94—100 (1996).
22) Kramer K.K., Zoelle J.T., Klaassen C.D., *Toxicol. Appl. Pharmacol.*, **141**, 1—7 (1996).