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# Effects of Intracerebroventricular Injection of Iron Dextran on the Iron Concentration and Divalent Metal Transporter 1 Expression in the Caudate Putamen and Substantia Nigra of Rats

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ABSTRACT

The cellular localization of DMT1 and its functional characterization suggest that DMT1 may play an important role in the physiological brain iron transport. But the regulation of DMT1 expression by iron in the brain is still not clearly understood. In this study, both the contents of ferric and ferrous iron as well as DMT1 expression were evaluated in CPu and SN after ICV of 500 μg iron dextran/rat/day for 3 or 7 days. It was found that the iron levels in CPu and SN were not altered obviously until ICV for 7 days. Immunohistochemistry results indicated that the expression of DMT1 (−IRE) in CPu and SN was not altered significantly after 3 days of ICV. Whereas the expression of DMT1 (−IRE) decreased significantly after 7 days of ICV when ferrous iron was increased significantly. Contrary to that of DMT1 (−IRE) in the same regions, there were no significant alterations in DMT1 (+IRE) expression in CPu and SN in spite of the existence of the altered iron levels, compared with that of control groups. The results demonstrate that DMT1 (−IRE) expression was correlated probably with brain iron levels; especially, its regulation was

Abbreviations used: CG = saline-injected control groups; CNS = central nervous system; CPu = caudate putamen; DCT1 = divalent cation transporter; DMT1 = divalent metal transporter 1; Ft = ferritin; ICV = intracerebroventricular injection; IG = iron dextran-injected groups; IHC = immunohistochemistry; IRE = iron-responsive element; Nramp2 = natural resistance associated macrophage protein 2; PBS = phosphate buffered saline; PD = Parkinson's disease; Slc11a2 = solute carrier family 11, member a2; SN = substantia nigra; Tf = transferrin; TfR = transferrin receptor; UTR = untranslated regions.

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correlated with ferrous iron (not ferric iron) in CPu and SN in adult rats, compared with those of saline-injected control rats. The effect of ferrous iron on the expression of DMT1 (−IRE) in the brain also suggests that it might play a major physiological role in brain iron uptake and transport, but further studies are needed to clarify these issues. Anat Rec, 292:225–233, 2009. 

**Key words: divalent metal transporter 1; intracerebroventricular injection; substantia nigra; caudate putamen; brain iron metabolism**

Iron is essential for the normal central nervous system (CNS) growth and development (Ke and Qian, 2003). In the brain, iron overload and iron-induced oxidative stress constitute a common mechanism that is involved in the development of neurodegenerations such as Parkinson's disease (PD) and Alzheimer's disease (Qian and Shen, 2001). Now a lot of evidences point out that the iron levels in basal ganglia and substantia nigra are higher in PD brains than in normal brains (Dexter et al., 1989; Martin et al., 1998; Griffiths et al., 1999; Wolozin and Golts, 2002; Moos and Morgan, 2004). Therefore, iron metabolism needs to be tightly regulated by various proteins that transport, sequester, and mobilize iron (De Silva et al., 1996).

Divalent metal transporter 1 (DMT1) is the first identified mammalian transmembrane iron transporter, and it is also known as natural resistance associated macrophage protein 2/divalent cation transporter/solute carrier family 11, member a2 (Nramp2/DCT1/Slc11a2) (Gruenheid et al., 1995; Andrews, 1999; Touret et al., 2003). DMT1 has been shown to transport a variety of divalent metals including $Fe^{2+}$ (Fleming et al., 1998; Harris et al., 1999; McKie et al., 2001). There are at least two different splice forms of DMT1: DMT1 (+IRE) mRNA containing iron-responsive element (IRE) in the 3′-UTR and DMT1 (−IRE) mRNA not containing a classical IRE (Gunshin et al., 1997; Lee et al., 1998).

Evidences showed that abundant DMT1 mRNA expresses in the striatum, substantia nigra, and other brain regions (Gunshin et al., 1997; Burdo et al., 2004; Knutson et al., 2004; Ke et al., 2005). An accumulation of iron occurs with age, and levels of DMT1 are correlated with iron staining in the monkey basal ganglia (Huang et al., 2004). Our previous data also showed that development can significantly affect brain iron and DMT1 expression (Ke et al., 2005). In the Belgrade rats with mutations in DMT1, brain nonheme iron concentrations were decreased compared with the controls (Burdo et al., 1999; Zywicke et al., 2002). In the neurons of the substantial nigra in PD, DMT1 is moderately high expressed that coincidentally correlates to the iron abnormal deposition in the same area (Andrews et al., 1999). Therefore, disruption of DMT1 expression may be involved in the increased iron accumulation in PD. In addition to PD, abnormally high level of iron in the brain has also been demonstrated in other neurodegenerative disorders (Swaiman, 1991; Qian and Wang, 1998; Aisen et al., 1999; Jellinger, 1999). These demonstrated that DMT1 expression is very important in CNS and is closely correlated with brain iron.

However, the effect of iron on DMT1 expression in the brain is still not well understood. Previous studies have been focused on the effect of iron status on the expression of DMT1 in peripheral organs, tissues, and cell lines (Wardrop and Richardson, 1999; Yeh et al., 2000; Gambling et al., 2001; Gunshin et al., 2001; Hubert and Hentze, 2002; Ke et al., 2003; Siddappa et al., 2003). They have produced controversial results because of the different isoforms of DMT1 in different organisms or tissues and have found an unexpected complexity of DMT1 expression and regulation. Our previous results (Ke et al., 2005) indicated that there was no significant alternation in DMT1 expression in the brain in spite of the existence of the altered iron levels after 6-week diet treatment. But this could not discern which form of iron (ferric or ferrous) was correlated with DMT1 expression more closely, nor could it confirm whether the expression of DMT1 had been altered during the long 6-week period.

Because of these puzzles and the putative contribution of DMT1 in the development of some neurodegenerative disorders, we further examined the effect of iron [intracerebroventricular injection (ICV)] on DMT1 expression in the brain with iron histochemistry and immunohistochemistry (IHC) methods.

## EXPERIMENTAL PROCEDURES

### Subjects

Male Sprague-Dawley rats (HEB LAC in Hebei Medical University, PRC), which weighed ~190 g at the beginning of the experiments, were divided into saline-injected control groups (CG) and iron dextran-injected groups (IG), and each group has six rats. They were housed in the same conditions as follows: temperature, 21°C–22°C; humidity, 51%; and a light controlled 12:12 hr light/dark cycle. Food was freely available. All rats were allowed at least 3 days to adapt to their living conditions before surgery. All animal experiments were performed in accordance with regulations for the administration of affairs concerning experimental animals (1997.539) by the State Science and Technology Commission of People's Republic of China.

### Surgery

All surgery was performed under aseptic conditions. Rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and placed in a stereotaxic instrument (SR-6N, Tokyo, Japan). Then, a 20-gauge, stainless steel guide cannula with its dummy cannula (Plastics One

Inc., Roanoke, VA) was placed 0.5 mm above the rat's left lateral ventricle and anchored to the skull using stainless steel screws and dental cement.

Rats were implanted with cannulae according to the following coordinates: rostral–caudal, −0.8 mm (0.8 mm posterior to bregma), medial–lateral, 1.5–2.0 mm from the midline, and dorsal–ventral, 3.2 mm from the surface of the dura. Animals were allowed 1 week to recover from surgery and were given penicillin (300,000 U/mL, 0.2 mL, i.m.) to prevent postoperative infection every day.

### Drugs and ICV Injection Procedure

Iron dextran (100 μg/μL) was purchased from Sigma, Inc. (St. Louis, MO). It was administered into the lateral ventricles of the IG rats in a volume of 5 μL in a 2-min period. The corresponding CG rats received the same amount of normal saline (0.9% NaCl) for the same time. IG rats were divided into two groups: IG3 group (ICV for 3 days) and IG7 group (ICV for 7 days), and so were CG rats (CG3 and CG7 groups, respectively). For infusions, the dummy cannula was replaced with an injector cannula attached to a Hamilton syringe, via PE 50 tubing.

### Brain Dissection and Slide Preparation Procedures

Rats were anesthetized by sodium pentobarbital (40 mg/kg, i.p.) in turn after a 24-hr interval to the final injection. Initially rats were perfused through the ascending aorta for a minimum of 5 min with ice-cold normal saline (0.9% NaCl), until the effluent from the right atrium was clear of blood, and then they continued to be perfused with 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.2–7.4). Both perfused IG and CG rats were decapitated, and their brains were removed from the same coordinates and continuously postfixed in the same fixative solution. After being rinsed in 0.1 M PB (pH 7.2–7.4), rat brains were dipped in 30% sucrose in 0.1 M PB for 2 days. Next, rat brain sections were cut coronally at 15 μm thickness on a freezing microtome. According to our previous reports (Ku et al., 1999), the sections containing caudate putamen (CPu; A6860–A7190 μm) and substantia nigra (SN; 1.8 mm to A2.2 mm) were attached to aminopropyltriethoxy silane-coated glass slides. Also, the sections containing CPu from IG rats and CG rats were mounted on the same glass slides to ensure the following reactions in identical conditions, and so were those of SN. They were stored at −20°C until processing.

### Iron Histochemistry

Iron was detected in cryostat sections of the brain using a modified Perl's staining (Smith et al., 1997; Calingasan et al., 1998; Roberts et al., 2001; Yu et al., 2001; Moos et al., 2002; Patel et al., 2002; Wang et al., 2002a). After rinsing three times in deionized water for 30 min, the sections were incubated at room temperature for 20 min in 1% $H_2O_2$ in 0.01 M phosphate-buffered saline (PBS) to quench endogenous peroxidase activity and then rinsed three times in deionized water for 30 min. After that, they were incubated at room temperature for 15 hr in a freshly made Perl's solution [1% potassium ferrocyanide in 1% aqueous hydrochloric acid for ferric iron detection or 7% potassium ferricyanide in 3% aqueous hydrochloric acid for ferrous iron detection] and subsequently rinsed three times (10 min each) in deionized water and developed in a diaminobenzidine (DAB) solution (DAB substrate kit SK-4100, Vector Laboratories), using half of the recommended DAB stock solution, for 15 min. Tissue sections were then rinsed thoroughly in distilled water and dehydrated through a series of graded alcohols (5 min each in 70% and 80%, followed by 10 min each in 90%, 95%, and 100% ethanol) before being immersed in xylene (twice for 10 min each). They were then coverslipped with Canada balata. Negative control sections were incubated in Perl's solution, but with PBS in place of potassium ferrocyanide/potassium ferricyanide. The control slides did not show any positive staining.

### Immunohistochemistry

Tissue was stained using the avidin:biotinylated enzyme complex method (Vector Laboratories). First, the sections were washed in 0.01 M PBS (pH 7.4). Endogenous peroxidase was blocked by 0.3% $H_2O_2$ in methanol for 25 min. To ensure same incubation conditions, the sections from CG and IG in one glass slide were placed in one drop of reaction solution by the following steps. Blocking with normal goat serum for 30 min at room temperature, the sections were incubated in a primary rabbit anti-rat DMT1 with IRE/without IRE antibody (ADI, San Antonio) with a 1:500 dilution for 24 hr at 4°C, followed by a secondary goat anti-rabbit serum (Vector Laboratories), and then in the avidin-biotinylated horseradish peroxidase complex (Vector Laboratories) for 60 min at 37°C. The sections were rinsed in PBS six times (5 min each) after every step. Lastly, the reaction was developed in DAB solution (DAB substrate kit SK-4100, Vector Laboratories) for 5 min. In iron histochemistry and IHC methods, it was kept same for the reaction period of sections with DAB strictly, and hence the glass slides were immersed in DAB solution in order, followed by termination of the reactions with distilled water orderly, too. In the same glass slides, the earlier sections contacted with DAB were rinsed with distilled water more early. Finally, the sections were serially submerged in increasing concentrations of ethyl alcohol, and then cleared and coverslipped as described earlier for iron histochemistry. Negative control sections were incubated in the same way, but with normal rabbit serum in place of the primary antibody, and showed absence of staining.

### Iron Histochemistry and IHC Analysis

Brain sections from the same coronal level were selected from IG and CG rats, and they were photographed with the same parameters using a Leica microscope (Leica DM IRB, Wetzlar GmbH, Germany) with a video camera (Leica DC 300F). Microscopic images of CPu and SN were captured in real-time provided by 1.20 version of Leica IM50 programme using Leica DC 300F (Leica Microsystems AG, Heerbrugg, Germany), and the exposure time was kept at 41.6 ms in both CG and IG groups. The average light intensity of the iron-stained and DMT1-stained cells in the images were analyzed and measured with the aid of Motic Digital Medical Image Analysis System (Motic Med 6.0, Motic China Group Co., Ltd).

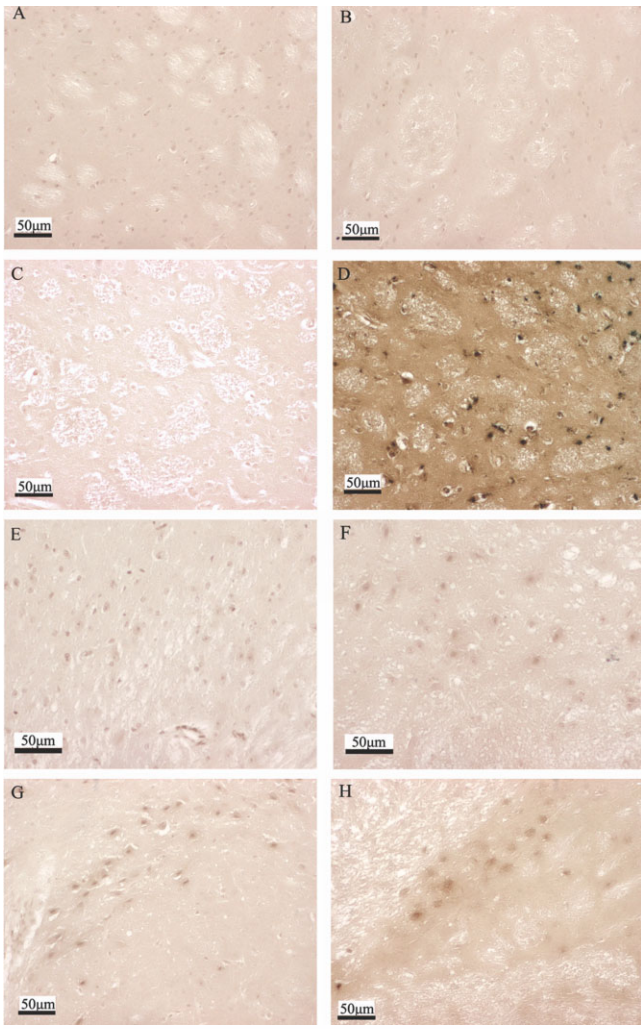


Fig. 1. The photomicrographs of ferric iron in CPu (**A–D**) and SN (**E–H**) after intracerebroventricular injection (ICV) of iron dextran for 3 (A,B,E,F) and 7 days (C,D,G,H). A,C,E,G: Saline-injected control group; B,D,F,H: Iron dextran-injected group; Scale bars: 50 μm.

### Statistical Analysis

The data are expressed as means ± SE. Differences between means were determined by Student's paired $t$ test. A probability value of $P < 0.05$ was considered statistically significant.

## RESULTS

### Iron Histochemical Staining for Ferric Iron in CPu and SN After ICV of Iron Dextran for 3 and 7 Days

Ferric iron staining studies with histochemical method in CPu and SN of CG and IG rats revealed ferric iron distribution (Figs. 1 and 2). Little staining of ferric iron was observed in CPu (Fig. 1A,C), while much more staining was observed in SN (Fig. 1E,G) in the saline-injection control rats. As is seen, iron granules were mainly restricted in the neurons and glial cells.

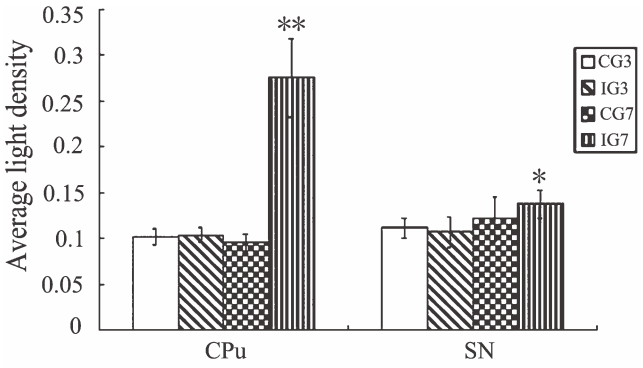


Fig. 2. The ferric iron status ($Fe^{3+}$) in CPu and SN after ICV of iron dextran. All data were expressed as mean ± SE. *$P < 0.05$ vs. CG; **$P < 0.01$ vs. CG.

After ICV of iron dextran for 3 days, no increase in ferric iron staining was detected in both CPu and SN (Fig. 1B,F). At 7 days postinjection of iron dextran into the lateral ventricle, an increase of ferric iron staining was observed in both CPu and SN (Fig. 1D,H). Data from image analysis showed that ferric concentrations in CPu and SN were increased significantly ($P < 0.01$ and $P < 0.05$, respectively) after iron dextran (500 μg/rat/day) injection into the lateral ventricle continuously for 7 days, compared with those of CGs (Fig. 2).

### Iron Histochemical Staining for Ferrous Iron in CPu and SN After ICV of Iron Dextran for 3 and 7 Days

Level of ferrous iron in CPu and SN was also determined using iron histochemistry in CG and IG rats (Figs. 3 and 4). Compared with the staining of ferric iron, much less staining to ferrous iron was observed in CPu (Fig. 3A,C) and SN (Fig. 3E,G), especially in CPu in saline-injected control rats. The cellular localization pattern of ferrous iron staining was similar to that of ferric iron.

After 3 days postinjection of iron dextran (500 μg/rat/day) into the lateral ventricle, no increase in ferrous iron staining could be detected in both CPu and SN (Fig. 3B,F), compared with that of the CGs. After ICV of iron dextran for 7 days, an increase of ferrous iron staining could be detected in both CPu and SN (Fig. 3D,H). Data from image analysis showed that ferrous iron concentrations in CPu and SN were both increased obviously ($P < 0.01$) after iron dextran injection into the lateral ventricle continuously for 7 days, compared with those of CGs (Fig. 4).

### IHC Staining for DMT (+IRE) in CPu and SN After ICV of Iron Dextran for 3 and 7 Days

In sections of IG and CG rat brains, the studies with IHC showed that DMT1 (+IRE) immunoreactivity showed dense staining in SN (Fig. 5E,G) and light staining in CPu (Fig. 5A,C). The neurons and glial cells were stained positive for DMT1 (+IRE), especially distinct in SN. The staining was concentrated in the cytoplasm of the neurons and the cell body of glial cells.

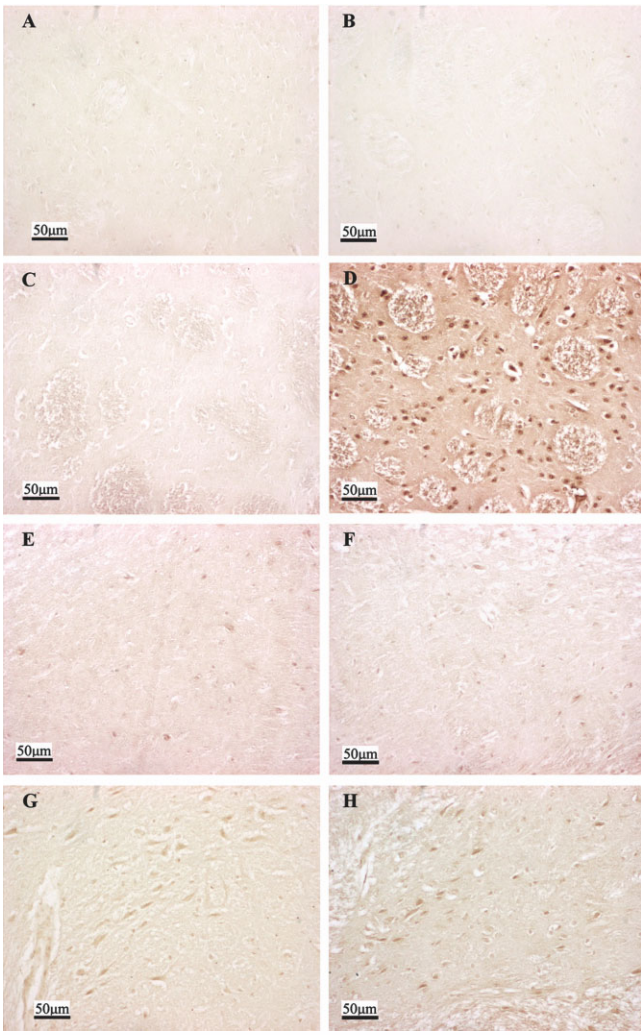


Fig. 3. The photomicrographs of ferrous iron in CPu (**A–D**) and SN (**E–H**) after ICV of iron dextran for 3 (A,B,E,F) and 7 days (C,D,G,H). A,C,E,G: Saline-injected control group; B,D,F,H: Iron dextran-injected group; Scale bars: 50 μm.

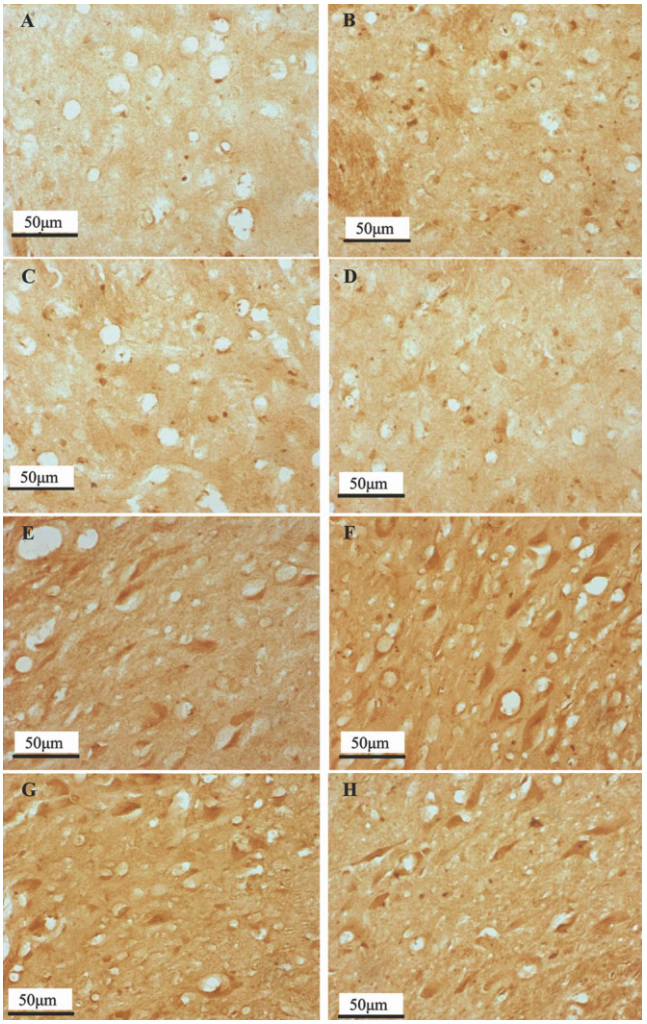


Fig. 5. The photomicrographs of DMT1 (+IRE) staining in CPu and SN after ICV of iron dextran. **A–D:** CPu; **E–H:** SN; A,C,E,G: Saline-injected control group; B,D,F,H: Iron dextran-injected group; A,B,E,F: injection for 3 days; C,D,G,H: injection for 7 days; Scale bars: 50 μm.

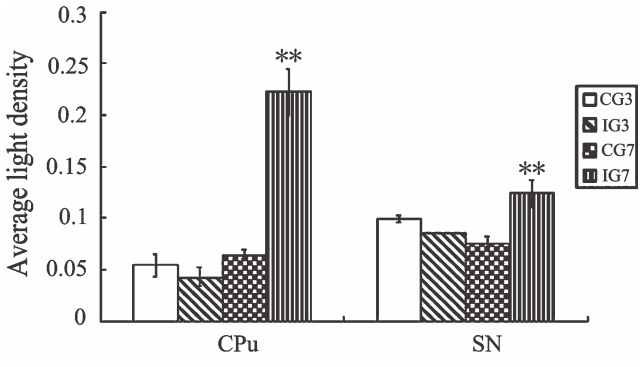


Fig. 4. The ferrous iron status ($Fe^{2+}$) in CPu and SN after ICV of iron dextran. All data were expressed as mean ± SE. $^{**}P < 0.01$ vs. CG.

From the image analysis, it was found that the average light density of DMT1 (+IRE) in CPu was increased significantly ($P < 0.01$) and not changed respectively after ICV of iron dextran (500 μg/rat/day) for 3 and 7 days. In SN, there were no significant alterations in the average light density of DMT1 (+IRE) after ICV of iron dextran (500 μg/rat/day) for 3 and 7 days, compared with that of CG (Fig. 6).

## IHC Staining for DMT1 (−IRE) in CPu and SN After ICV of Iron Dextran for 3 and 7 Days

Figure 7 showed the immunoactive staining of DMT1 (−IRE) in IG and CG rats. Similar immunolocalization pattern of DMT1 (−IRE) staining was observed in CPu and SN, and SN (Fig. 7E,G) was still densely stained for DMT1 (−IRE), while less dense staining was observed

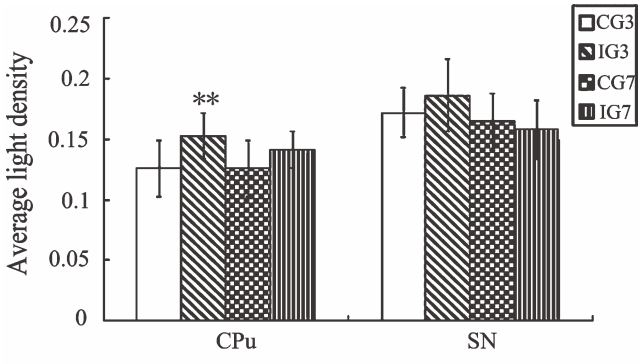


Fig. 6. The expression of DMT1 (+IRE) in CPu and SN after ICV of iron dextran. All data were expressed as mean ± SE. $^{**}P < 0.01$ vs. CG.

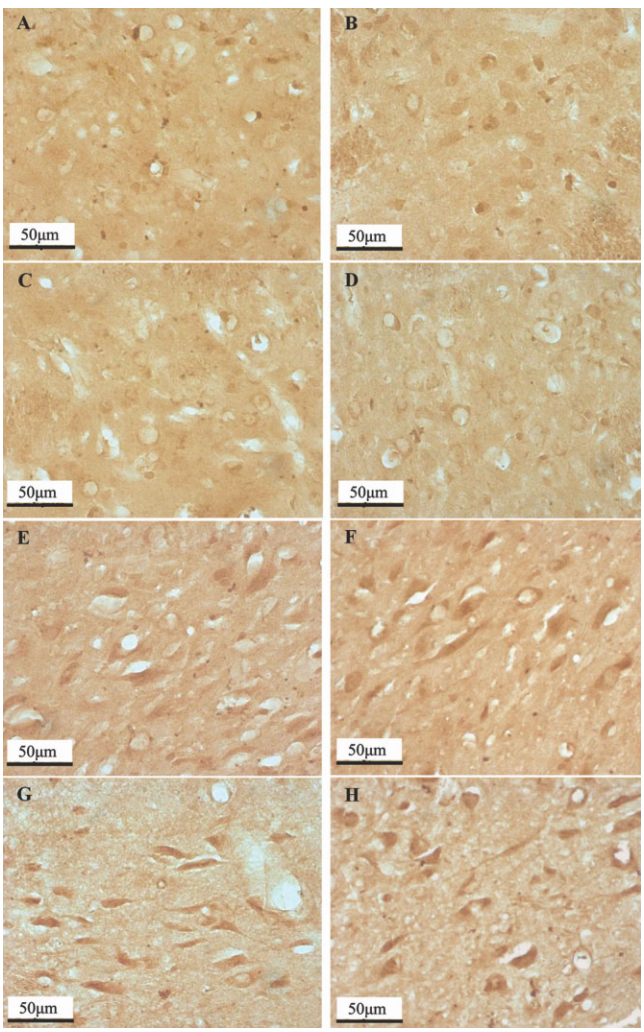


Fig. 7. The photomicrographs of DMT1 (−IRE) staining in CPu and SN after ICV of iron dextran. **A–D:** CPu; **E–H:** SN; A,C,E,G: Saline-injected control group; B,D,F,H: Iron dextran-injected group; A,B,E,F: injection for 3 days; C,D,G,H: injection for 7 days; Scale bars: 50 μm.

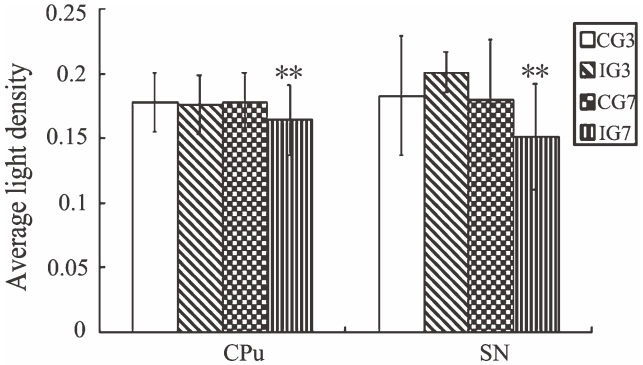


Fig. 8. The expression of DMT1 (−IRE) in CPu and SN after ICV of iron dextran. All data were expressed as mean ± SE. $^{**}P < 0.01$ vs. CG.

in CPu (Fig. 7A,C). Neuronal and glial cell bodies showed strong positive staining in the SN.

After ICV of iron dextran for 3 days, there were no alterations in the staining of DMT1 (−IRE) in CPu and SN, compared with that of CG. At seventh day postinjection of iron dextran, a decrease in the staining of DMT1 (−IRE) was detected. From the image analysis, it was found that there were significant decreases in the average light density of DMT1 (−IRE) in CPu and SN after iron dextran (500 μg/rat/day) injection into the lateral ventricle for 7 days ($P < 0.01$), compared with that of CG (Fig. 8).

## DISCUSSION

The present study investigated the effect of iron (ICV) on iron contents as well as DMT1 expression in CPu and SN. Results showed that iron dextran could alter brain iron levels (both ferric and ferrous). Moos and Morgan (1998) also found the similar alterations, and they demonstrated that, in CSF of adult rats, $^{59}$Fe was decreased to only 2.5% of the initial dose following its injection as a [$^{59}$Fe-$^{125}$I] transferrin (Tf) conjugate into the ventricular system for 4 hr, and that in brain parenchyma, ~18% of ICV-injected $^{59}$Fe was retained after 72 hr. To our knowledge, the alterations of ferric and ferrous iron content in different brain regions after ICV of iron was first systematically studied. We found that brain iron content was not altered after ICV of iron dextran (500 μg/rat/day) for 3 days. At seventh day postinjection, significant increases in ferric and ferrous iron were observed, compared with those of CG rats. In addition, an increase in ferric iron was detected, and no increase in ferrous iron level was observed in cerebral cortex after 3 days of injection, while the ferrous iron level was increased significantly when the level of ferric iron was increased to a very high level after iron injection for 7 days (data not shown). This implied that ferrous iron level was elevated with more and more iron entering the brain tissues.

Our previous investigations (Ke et al., 2005) have shown that there was no significant alternation in DMT1 (+IRE) and (−IRE) mRNA expression and protein content in rat cortex, hippocampus, striatum, and substantia nigra in spite of the existence of the altered iron levels in these regions after 6-weeks' diet treatment.

It is known that the distribution of iron in the brain is heterogeneous. Moreover, Moos and Morgan (1998) reported that $^{59}$Fe was retained in the adult brain in distinctly different degrees following its injection as a [$^{59}$Fe-$^{125}$I] Tf conjugate into the ventricular system, and their results showed that $^{59}$Fe was confined to the ventricular surface and meningeal areas, whereas gray matter regions at distances more than 2–3 mm from the ventricles and the subarachnoid space were unlabelled. Therefore, the relationship between DMT1 expression and iron in the brain needs to be investigated subtly from brain regions and methods. Our present experiment selected a more direct iron administration method—ICV of iron dextran. Furthermore, ferric and ferrous iron content was respectively determined in CPu (near lateral ventricle) and SN with iron histochemistry, and the expression of DMT1 was examined with IHC in the same regions after ICV of iron dextran. Data showed that there was no significant alteration in the expression of DMT1 (+IRE) in the two regions in spite of the significantly increased iron levels (both ferrous and ferric) in CPu and SN after ICV of iron dextran for 7 days. But the expression of DMT (−IRE) was decreased significantly in both CPu and SN at 7 days postinjection of iron dextran. These results imply that DMT1 (−IRE), not DMT1 (+IRE), is correlated with iron in the two regions under our experimental conditions.

The present results seem contrary to our previous results of no alterations of DMT1 (−IRE) expression upon the obvious alterations of iron after 6-weeks' diet treatment. This is probably due to the following reasons: First, the methods of increasing the iron status in the brain are different; Second, the percentage of brain iron elevation in our previous study is smaller (i.e., $<20\%$) than what was found in this study, which could not be high enough to alter DMT1 expression, and perhaps the regulation of transferrin receptor (TfR) was sufficient. Finally, in previous study, the increased iron was the total iron in brain regions determined using a graphite furnace atomic absorption spectrophotometer, and in this study it could be distinguished to ferric or ferrous using iron histochemistry. The expression of DMT1 (−IRE) was mainly connected with ferrous, not with ferric iron. After injection of iron dextran into the lateral ventricle for 3 days, no increase of ferric and ferrous iron was detected in CPu and SN, and no alterations in the expression of DMT1 (−IRE) were observed either; While after iron injection into the lateral ventricle for 7 days, the contents of ferric and ferrous iron in CPu and SN were increased obviously, while the expression of DMT1 (−IRE) was decreased obviously. Furthermore, we have also detected the iron contents and expression of DMT1 in cerebral cortex. A similar pattern of iron staining was also observed in this region: At third postinjection, the contents of ferrous iron were not changed, and ferrous iron was increased obviously at seventh postinjection. While the content of ferric was already increased obviously at third postinjection, its contents became more at seventh postinjection of iron dextran (data not shown). However, the expression of DMT1 in cerebral cortex was not changed significantly until the ferrous iron was increased at seventh postinjection. It seemed like that expression of DMT1 was correlated closely with the level of ferrous iron, and its expression was changed according to the changes of ferrous iron level. These results imply that DMT1 (−IRE) in CPu and SN was mainly correlate with ferrous, and not ferric iron in rat brain.

However, there was an increase in the expression of DMT1 (+IRE) in CPu and SN we examined at 3 days postinjection of iron, which might be due to a contemporary response of DMT1 to the increased iron (ICV) in brain parenchyma. In addition, the two isoforms of DMT1 might have different responses to iron status: DMT1 (+IRE) might be correlated mainly with the iron level in a short period of time, and DMT1 (−IRE) might play more important roles in iron transport with the more iron dextran injected into the lateral ventricle.

Studies showed that DMT1 expression and translation in response to iron status in different organs or tissues might be different. The expression of DMT1 in the small intestine is negatively regulated by iron status (Tandy et al., 2000; Trinder et al., 2000; Yeh et al., 2000; Gunshin et al., 2001). However, DMT1 expression in hepatocytes is positively regulated by iron at the posttranscriptional level (Trinder et al., 2000). DMT1 expression in the kidney is only slightly increased upon deprivation of dietary iron (Canonne-Hergaux and Gros, 2002). In LMTK-, RAW264.7 and J774 cells, iron has no significant effect on DMT1 expression (Wardrop and Richardson, 1999, 2000). Gunshin et al. (2001) reported that the response of DMT1 (+IRE) to iron was tissue specific and cell type specific. The cell and tissue specificity of DMT1 expression also occurred in perinatal rat brain, and it was found that iron deficiency increased the expression of DMT1 in hippocampus and cerebral cortex, but had no effect on other regions (Siddappa et al., 2003). This demonstrated that DMT1 expression was regulated by iron in a tissue- and cell-specific way. Our present study found that DMT1 (−IRE) expression in CPu and SN was decreased significantly in response to the increased ferrous iron levels after ICV of iron dextran for 7 days. These indicate that the expression of DMT1 (−IRE) is negatively correlated with the alteration in nonheme ferrous iron status.

Previous researches have shown that DMT1 might play an important role in iron transport across the apical surface of intestinal enterocytes (Tandy et al., 2000; Trinder et al., 2000), and that it might be involved in translocation of iron from endosome to cytosol under physiological conditions (Su et al., 1998; Gruenheid et al., 1999; Tabuchi et al., 2000). It was reported that DMT1 might have a role in Tf-dependent iron uptake in some types of cells (Wardrop and Richardson, 1999). In the brain, the existence of DMT1 and its functional characterization suggest that DMT1 might play an important physiological role in iron homeostasis in the brain (Gunshin et al., 1997; Andrews, 1999; Andrews et al., 1999; Burdo et al., 1999; Williams et al., 2000; Wang et al., 2001, 2002b). However, functional information is still lacking. In general, TfR and ferritin (Ft) play a key physiological role in iron metabolism, and they could respond to as well as to control the changes in iron levels in the cells and tissues. In our previous study, no significant alternation was found in DMT1 expression in cortex, hippocampus, striatum, and substantia nigra of the high-iron or low-iron adult rats. The finding, plus the fact that DMT1 might pump other metals in the brain besides iron, seemed to imply that DMT1 might not have a primary regulatory role. But

the further study on the issue found that the expression of DMT1 (−IRE) was decreased significantly in CPu and SN examined because of the notable elevation of intracellular ferrous iron. This fact confirmed that the uptake of ferrous iron was mediated by DMT1 and that DMT1 (−IRE) might play a more critical regulatory role in brain cell iron uptake.

In summary, our results showed that ICV of iron dextran could alter brain iron levels (both ferric and ferrous iron). In addition, the content and speed of iron (ICV) were altered more quickly than that of high-diet feeding after limited period. We found that the expression of DMT1 (+IRE)/(−IRE) detected in CPu and SN was correlated with brain iron content, especially ferrous iron. We also found that the expression of DMT1 (−IRE) was negatively correlated with intracellular ferrous iron in adult rats. The two isoforms of DMT1 are both important in brain iron uptake, and DMT1 (−IRE) is the main isoform to play more significant and critical physiological function in brain iron metabolism.

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