

Disruption of the CaMKII/CREB Signaling is Associated with Zinc Deficiency-Induced Learning and Memory Impairments

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Abstract Many studies have shown that zinc deficiency not only retards growth, but also affects several brain functions, including learning and memory. However, the underlying mechanism of impaired hippocampus-dependent learning and memory under zinc deficiency is poorly understood. In this study, young mice were fed a zinc-deficient diet (0.85 ppm) for 5 weeks. Morris water maze result showed that zinc deficiency results in spatial learning impairment. We then examined whether zinc depletion-induced learning and memory defects are associated with changes in signaling molecules essential for the expression of long-term potentiation. Immunoblot results showed that the protein levels of calmodulin (CaM), phosphorylated CaM-dependent protein kinase II (CaMKII), and phosphorylated cAMP-responsive element binding protein (CREB) were significantly reduced, whereas the total protein levels of CaMKII and CREB did not change in the zinc-deficient hippocampus. Thus, we provide a previously unrecognized mechanism whereby zinc deficiency impairs hippocampal learning and memory, at least in part, through disruption of the CaM/CaMKII/CREB signaling pathway.

Keywords Calmodulin-dependent protein kinase II · cAMP-responsive element binding protein · Long-term potentiation · Morris water maze · Neurogranin

Introduction

The hippocampus is a critical brain region for learning and memory functions. It has been reported that the hippocampus is the richest zinc-containing area in the brain, and large amounts of loosely bound zinc ions are present in the synaptic vesicles in the giant boutons of hippocampal mossy fibers (Frederickson et al. 2005). The accumulation of zinc in synaptic vesicles is associated with a vesicular transmembrane protein, zinc transporter 3 (ZnT3), which can transport zinc ions into synaptic vesicles from cytosol (Palmiter et al. 1996). During synaptic transmission, vesicular zinc is released into the synaptic cleft to serve as a neuromodulator to modulate different types of receptors, including the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor, *N*-methyl-D-aspartate (NMDA) and γ -aminobutyric acid receptors and voltage-gated ion channels (Smart et al. 2004; Takeda et al. 2004). Interestingly, several studies have shown that the induction of mossy fiber long-term potentiation (LTP) can be inhibited after interaction of the synaptic zinc with zinc chelators (Lu et al. 2000; Takeda et al. 2008a). These results suggest that synaptically released zinc is involved in the induction of LTP in the hippocampus.

Epidemiological, clinical, and animal studies have shown that dietary zinc deficiency not only retards growth, but also induces learning and memory defects in mammals (Golub et al. 1995; Takeda 2000; Takeda et al. 2008a). Dietary zinc deficiency can cause a decrease in zinc concentration in the hippocampus (Takeda et al. 2008b; Gao et al. 2009), and result in learning impairment (Keller et al. 2000; Takeda et al. 2000, 2008a). For example, with 17-arm radial maze tests, deficits in working memory were found in the offspring when pregnant rats were fed zinc-deficient diets and throughout lactation (Halas et al. 1983, 1986). The adult rats were fed with zinc-deficient diets for

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4 weeks, the learning behavior, tested by passive avoidance performance, was significantly impaired (Takeda 2000). However, a recent study from the same group has shown that, with a short period (2 weeks) zinc deprivation, young adult mice did not show Schaffer collateral LTP changes revealed by the averaged field-excitatory postsynaptic potential (f-EPSP) after stimulation, and there was also no significant difference in learning behavior between the control and the zinc-deficient mice, revealed by Morris water maze tests (Takeda et al. 2008b). In addition, a study with ZnT3 knockout mice has shown that disruption of the ZnT3 gene results in a lack of synaptic vesicular zinc in hippocampal mossy fibers, but does not impair spatial learning and memory functions (Cole et al. 2001). Using a severe dietary zinc-deficient mouse model, we have recently reported that chronic treatment with a zinc-deficient diet (0.85 ppm Zn) for 5 weeks results in reduction in hippocampal neurogenesis and increases neuronal apoptosis, indicating that zinc deficiency is associated with destroying structural plasticity in the hippocampus (Gao et al. 2009). Taken together, these data suggest that zinc deficiency-induced learning and memory impairments are complicated, and there may be several cellular and molecular mechanisms responsible for the defects of learning and memory with zinc deficiency, rather than the reduction in vesicular zinc directly affecting LTP formation in the hippocampus.

Hence, in this study, we hypothesized that zinc deficiency may impair cell signal pathways related to LTP induction in the hippocampus. Since several lines of evidence have confirmed that the calmodulin (CaM)-dependent protein kinase II (CaMKII)/cAMP-responsive element binding protein (CREB) signaling pathway plays a key role in synaptic plasticity and learning and memory functions (Impey et al. 1996; Mayford et al. 1996; Silva et al. 1998; Barco et al. 2002; Kida et al. 2002; Lonze and Ginty 2002; Miller et al. 2002), and zinc can modulate the activity of several key molecules in this pathway (Lengyel et al. 2000). We therefore examined the possible mechanisms of impairing learning and memory, by analyzing the expression levels of key proteins in the CaMKII/CREB pathway, in the zinc-deficient mouse hippocampus.

Materials and Methods

Animals and Zinc-Deficient Diet

All experimental procedures were in agreement with the rules for experimental animals at China Medical University, in accordance with the criteria described in the *NIH Guide for the Care and Use of Laboratory Animals*. Three-week-old CD-1 mice, weighing about 13 g, were purchased

from Vital River Laboratory Animal Technology (Beijing, China). They were maintained in stainless steel cages, which were washed with 0.5% EDTA before use. Mice were randomly assigned to one of the two dietary groups ($n = 20$ in each group), a control group and a zinc-deficient group, which were fed 5 weeks with zinc-adequate and zinc-deficient diets, respectively. The zinc content of the zinc-adequate control and zinc-deficient diets was 30 and 0.85 ppm (Egg white-based AIN-76A, Research Diets Company, USA), respectively. All mice were allowed to consume purified, deionized water ad libitum.

Morris Water Maze

The Morris water maze tests were carried out in a circular tank (120-cm inner diameter and 40-cm in height), equipped with a digital pick-up camera 180 cm above the water surface to monitor the animal behavior and a computer program for data analysis (ZH0065, Zhenghua Bio-equipments, China). Tap water, 48-cm deep, was made opaque by the addition of black non-toxic paint and maintained at 25°C, and a translucent acrylic platform (8 cm in diameter) was placed in the center of the southeast quadrant. During training, the escape platform was exposed 1.5 cm above the water surface to guide the animals how to get out of the water. After mice were trained and ready for testing, the escape platform was placed just below the surface of the water (hidden platform) or removed from the tank. The visible platform training, navigation test, and probe trial were carried out as previously described with few modifications (Qing et al. 2008). Briefly, mice ($n = 7$ in each group) were trained individually for 2 days (3 contiguous trials, with an inter-trial interval of 30 min) to find the visible platform. On days 3 to 7, the hidden platform was prepared for the place navigation test, and each mouse was subjected to three trials per day with an inter-trial interval of 1 min from the farthest starting position. For each trial, the latency to escape to the hidden platform and the path length were recorded. Twenty-four hours after the last hidden platform test (the 8th day), the hidden platform was removed for the probe trial. The number of times the animal crossed the center of the southeast quadrant (where the hidden platform was previously located) with an interval of 1 min was recorded. Finally, data for the escape latency, the path length and the number of passing times between the zinc deficient and the control groups were analyzed statistically.

Immunohistochemistry

Mice ($n = 4$ in each group) were transcardially perfused with saline followed by 4% paraformaldehyde. The brains were dissected and immersed in the same fixative at 4°C.

The samples were then dehydrated and embedded in paraffin. Serial 6- μ m coronal sections were cut using a microtome. The routine ABC method was used to detect the distribution of p-CaMKII and p-CREB in mouse hippocampus. Briefly, sections were dewaxed, rehydrated, and treated with 0.1-M phosphate buffered saline (PBS) containing 10% methanol and 3% hydrogen peroxide (H_2O_2) for 10 min. After rinsing with PBS, the sections were treated by 5% bovine serum albumin (BSA) for 1 h, and then incubated overnight with the rabbit anti-phospho-CaMKII (Thr286) (1:100, Cell Signal) and rabbit anti-phospho-CREB (Ser133) (1:100, Cell Signal) antibodies, respectively, at 4°C. After rinsing, the sections were incubated in biotinylated goat anti-rabbit IgG (1:200) for 2 h at room temperature. After rinsing, the avidin–biotin-peroxidase complex (1:100, ABC kit, Elite Vector) was applied for 1 h at room temperature. The sections were immersed in 3,3-diaminobenzidine with 0.0033% H_2O_2 for about 10 min. After rinsing with distilled water, the sections were mounted and examined with a light microscope. Control sections were incubated with 5% BSA instead of the primary antibody. Subsequent incubations were as described above.

Western Blotting

Mice ($n = 7$ in each group) were killed by decapitation. Hippocampal fragments were minced and homogenized in cold lysis buffer overnight at 4°C. The lysates were collected, centrifuged at 12,000 rpm for 30 min, and quantified for total proteins using a UV 1700 PharmaSpec ultraviolet spectrophotometer (Shimadzu, Japan). The supernatant (soluble subcellular fraction) was removed, divided into aliquots, and stored at -80°C .

Thirty micrograms total protein of each sample was subjected to SDS-PAGE using 10% gradient Tris/glycine gels. Next, proteins were transferred to polyvinylidene difluoride membranes (Millipore, CA). After being blocked in 5% fat-free milk for 1 h, blots were incubated with the following primary antibodies: mouse anti-CaM (1:500, Upstate), rabbit anti-CaMKII (1:1000, Cell Signal), rabbit anti-phospho-CaMKII (Thr286) (1:1000), rabbit anti-CREB (1:1000, Cell Signal), rabbit anti-phospho-CREB (Ser133) (1:1000), rabbit anti-CaN (1:500, Chemicon), goat anti-Ng (1:500, Santa Cruz), and mouse anti-GAPDH (1:10000, KC-5G5, Kang Chen, CA), respectively, overnight at 4°C. Then, the membranes were washed and incubated with HRP-conjugated second antibody (1:5000, Santa Cruz) for 2 h at room temperature. Immunoreactive bands were visualized by the SuperSignal West Pico chemiluminescent substrate (Pierce, CA), and analyzed by ChemDoc XRS with Quantity One software (BioRad, USA).

Statistical Analysis

All statistical analyses were carried out using a program SPSS 13.0. All values were expressed as mean \pm standard deviation (SD). Comparisons were carried out by Student's *t* test and $P < 0.05$ was considered significant.

Results

Dietary Zinc Deficiency Induces Spatial Memory Deficits in CD-1 Mice

To investigate whether zinc-deficient diet treatment affects learning and memory in CD-1 mice, Morris water maze tests were performed after mice were fed zinc-deficient diet 5 weeks starting at the age of 3 weeks. The behavioral tests consisted of 2 days of visible platform tests, 5 days of hidden platform tests, and a probe trial 24 h after the last hidden platform test. In the visible platform tests, zinc deficient and control mice had similar escape latency (53.63 ± 10.74 vs. 51.96 ± 12.19 s; $P > 0.05$; Fig. 1a) and path length (11.85 ± 4.03 vs. 12.59 ± 4.84 m; $P > 0.05$; Fig. 1b), which indicated that zinc deficiency did not significantly affect motility or vision in our mouse model. However, in the place navigation (hidden platform) tests, zinc-deficient mice showed a longer escape latency and a longer path length before swimming onto the hidden platform when compared with the normal diet control mice. From the 2nd to 5th day, the escape latency of the zinc-deficient group and control group was 57.00 ± 10.89 vs. 42.15 ± 6.08 s ($P < 0.05$), 49.14 ± 8.26 vs. 31.76 ± 8.11 s ($P < 0.05$), 49.52 ± 6.80 vs. 33.96 ± 5.57 s ($P < 0.05$), 42.86 ± 10.96 vs. 23.56 ± 6.34 s ($P < 0.01$), respectively (Fig. 1c), and the path length of the zinc-deficient group and control group was 13.36 ± 2.57 vs. 10.32 ± 2.53 m ($P < 0.05$), 11.86 ± 2.64 vs. 8.27 ± 1.33 m ($P < 0.01$), 11.88 ± 1.77 vs. 7.94 ± 3.26 m ($P < 0.01$), 11.75 ± 2.44 vs. 6.42 ± 3.09 m ($P < 0.01$), respectively (Fig. 1d). Furthermore, the probe trial showed that the number of times the mice traveled into the center of the southeast quadrant, where the hidden platform was previously placed, was significantly less for zinc-deficient mice compared with controls (1.14 ± 0.69 vs. 2.67 ± 1.22 ; $P < 0.05$; Fig. 1e). Taken together, these data suggest that zinc deficiency can cause spatial memory impairments in CD-1 mice.

Zinc Deficiency Affects the Levels of Key Signaling Molecules Involved in Learning and Memory in the Hippocampus

To analyze the possible changes of LTP-related molecules in the dietary zinc-deficient mouse hippocampus, we

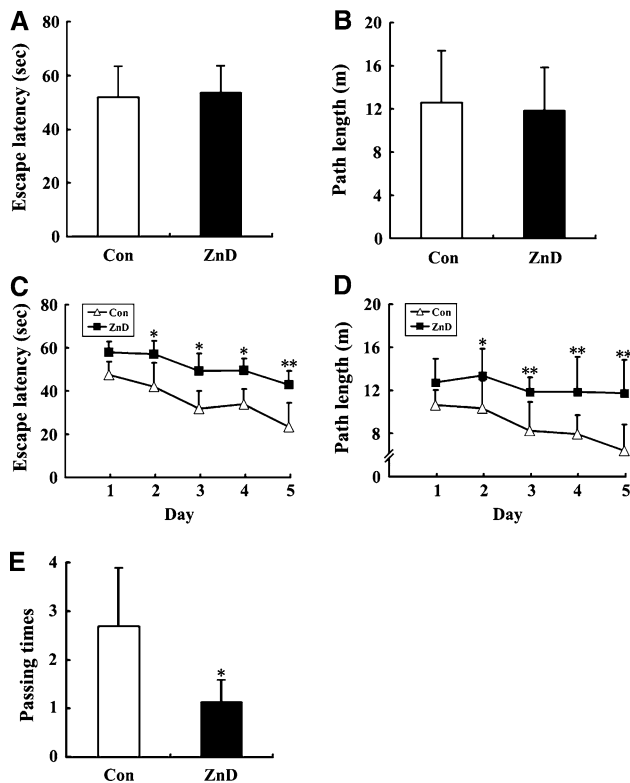


Fig. 1 Zinc deficiency causes spatial memory deficits in CD-1 mice. **a, b** Visible platform tests showed that, during the first 2 days, the zinc-deficient mice and controls exhibited a similar escape latency (**a**) and swimming distances (**b**) before escaping onto the visible platform. **c, d** In the hidden platform tests, zinc-deficient mice exhibited a longer escape latency (**c**) and a longer swimming length (**d**) before escaping onto the hidden platform from the 2nd to 5th day. **e** A probe trial showed that the zinc-deficient mice traveled into the center of the southeast quadrant, where the hidden platform was previously placed, significantly less times than controls. * $P < 0.05$, ** $P < 0.01$; $n = 7$

focused on detecting CaM/CaMKII/CREB signaling proteins with Western blot analysis. As shown in Figs. 2, 3, and 4, all analyzed proteins were detected in bands located at 17 kDa for CaM, 50 kDa for CaMKII and for p-CaMKII (Thr286), 18 kDa for Ng, 61 kDa for CaN, and 43 kDa for CREB and p-CREB (Ser 133), matching the predicted molecular mass of these proteins.

Among the CaM/CaMKII/CREB pathway, CaMKII has provided the most convincing evidence for being a key mediator in regulating the expression of LTP and memory formation (Wayman et al. 2008), and zinc can directly activate CaMKII independently of calcium and CaM (Lengyel et al. 2000). Therefore, we first examined whether zinc deficiency could affect the activity of CaMKII in the hippocampus. As shown in Fig. 2, immunohistochemical results showed that the intensity of p-CaMKII, the active form of CaMKII, was markedly decreased in CA1, CA3, and DG of the zinc-deficient mice (Fig. 2a–f). Immunoblot

analyses showed that the expression level of p-CaMKII was significantly lower in the hippocampus of zinc-deficient mice than that of control mice (Fig. 2g). Quantification of p-CaMKII revealed that zinc deficiency significantly reduced the basal level of p-CaMKII to $83.47 \pm 3.91\%$, compared with the control group ($P < 0.01$, Fig. 2g). In contrast, there was no significant change in total CaMKII protein in the zinc-deficient hippocampus ($P > 0.05$, Fig. 2g).

Then, we examined whether the upstream molecules in the CaMKII pathway were changed in the zinc-deficient mouse hippocampus. CaM, a high-affinity cytoplasmic calcium-binding molecule, is critical for CaMKII activation, resulting in CaMKII auto-phosphorylation. Zinc has been reported to affect the calcium/CaM binding to CaMKII (Lengyel et al. 2000). In hippocampal homogenates, our immunoblot results demonstrated that the CaM protein level in the zinc-deficient group was reduced $16.53 \pm 5.09\%$ when compared with the control group ($P < 0.01$, Fig. 3a). Ng and CaN are involved in modulation of the calcium/CaM-mediated pathway and p-CaMKII activation, respectively. Therefore, we measured Ng and CaN protein levels by immunoblotting in the zinc-deficient hippocampus. We found that the hippocampal Ng protein level in zinc-deficient mice was reduced to $66.11 \pm 15.17\%$, compared with control mice ($P < 0.05$, Fig. 3b). However, compared with the control group, no significant change in CaN level was detected in zinc-deficient mice ($P > 0.05$, Fig. 3c).

Finally, we assessed the effect of zinc deficiency on the expression level of CREB, an important downstream protein of CaMKII signaling pathway. It is known that, in the nucleus, activated CaMKII phosphorylates CREB by its phosphorylation at Ser133 (Sheng et al. 1991; Shaywitz and Greenberg 1999), resulting in activation of gene transcription and LTP maintenance (Montminy et al. 1990; Meyer and Habener 1993; Tropea et al. 2008). Our immunohistochemical results showed that p-CREB immunoreactivity was markedly reduced in hippocampal CA1, CA3, and DG of zinc-deficient mice (Fig. 4a–f). Furthermore, Western blot results demonstrated that zinc deficiency significantly reduced the basal level of p-CREB, the active form of CREB ($P < 0.01$, Fig. 4g), but the level of total CREB remained unchanged ($P > 0.05$, Fig. 4g). The ratio of p-CREB/CREB was reduced in the zinc-deficient group when compared with the control group ($P < 0.05$, Fig. 4g).

Discussion

In this study, we used a severe dietary zinc deficiency mouse model to examine the changes in learning and memory behavior, and LTP-related signaling pathway in

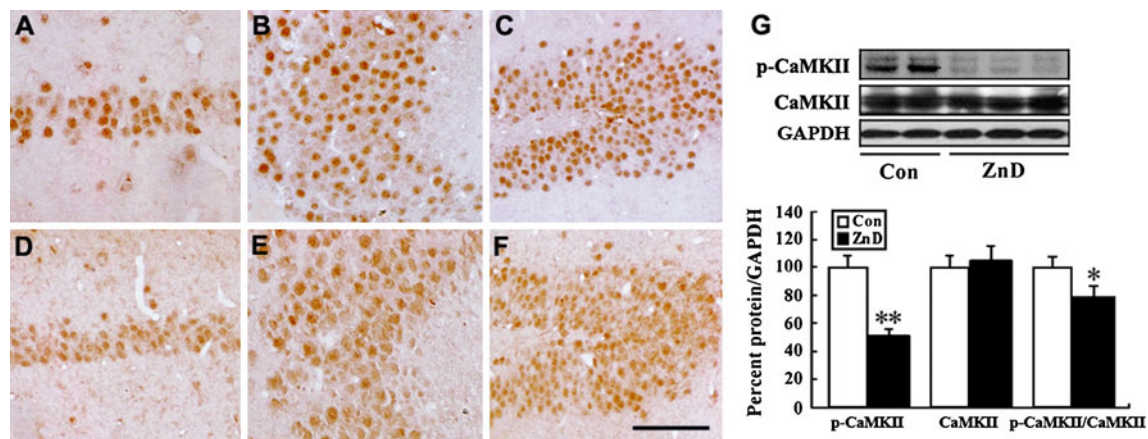


Fig. 2 Zinc deficiency induced changes in the expression levels of p-CaMKII protein in the hippocampus. **a–f** p-CaMKII immunoreactivity in the hippocampal CA1 (**a, d**), CA3 (**b, e**), and dentate gyrus (**c, f**) of controls (**a–c**) and zinc-deficient mice (**d–f**). There was a marked reduction in the intensity of p-CaMKII in the hippocampus of zinc-deficient mice. Scale bar = 50 μ m. **g** Representative images of

immunoblots are shown with antibodies against p-CaMKII and CaMKII, respectively. GAPDH was used as a loading control. Zinc deficiency significantly reduced the expression level of p-CaMKII protein in the hippocampus. In contrast, there was no obvious difference in total CaMKII between zinc-deficient (ZnD) and control (Con) groups. ** $P < 0.01$; $n = 4$

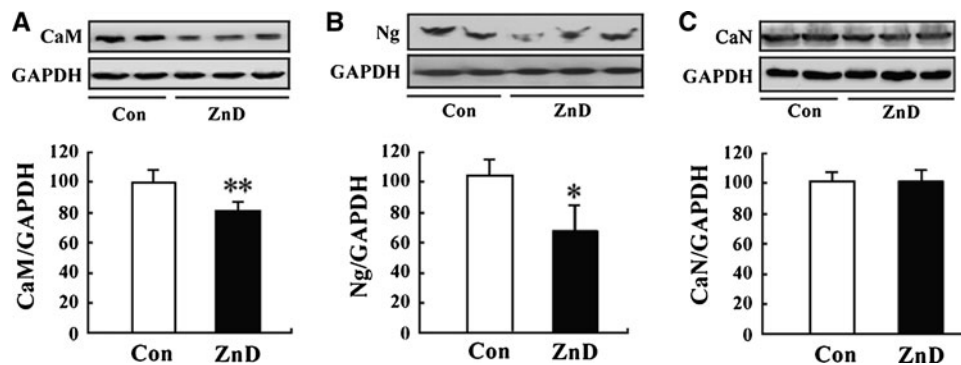


Fig. 3 Expression levels of CaM, Ng, and CaN proteins in the zinc-deficient mouse hippocampus. **a** Compared with the control (Con), the protein level of CaM was significantly reduced in the zinc-deficient group (ZnD). **b** The expression level of Ng was markedly reduced in

the zinc-deficient (ZnD) group when compared with the control (Con). **c** No obvious difference in CaN level was found between the zinc-deficient (ZnD) and the control (Con) groups. GAPDH was used as a loading control. * $P < 0.05$, ** $P < 0.01$; $n = 7$

the hippocampus. With the same zinc deficiency mouse model, we have previously reported that a reduced zinc concentration was found in the hippocampus in the zinc-deficient mouse brain, detected by zinc-fluorescence (TSQ) and autometallography (Gao et al. 2009). Our Morris water maze result showed that the zinc-deficient mouse exhibits defects in memory behavior. This is consistent with previous reports showing that dietary zinc deficiency might be involved in the damage to learning and memory in the hippocampus (Takeda et al. 2000). Since intracellular zinc is necessary for many enzyme activities and protein functions associated with signal transduction and gene expression (Vallee and Falchuk 1993), it is reasonable to speculate that zinc deficiency-induced impairments of hippocampus-dependent learning and memory function might be due to the disruption of the LTP-related signaling cascade. With our mouse model, we identified a new mechanism

whereby dietary zinc deficiency impairs learning and memory function mainly through disruption of the CaM/CaMKII/CREB signaling pathway in the hippocampus.

It has been reported that AMPA/kainite receptors elicit NMDA receptor activation and result in zinc (in addition to calcium) influx to postsynaptic neurons, which is believed to be involved in LTP induction (Weiss et al. 1989; Sensi et al. 1997; Takeda et al. 2007). LTP in the hippocampus is considered to be important for elucidating the molecular mechanism of learning and memory (Bliss and Collingridge 1993; Miyamoto and Fukunaga 1996; Fukunaga and Miyamoto 2000; Hudmon and Schulman 2002a; Costa-Mattioli et al. 2005; Gubellini et al. 2005; Worgotter and Porr 2005; Miyamoto 2006; De Roo et al. 2008). Calcium enters the postsynaptic neurons through AMPA and NMDA receptors and calcium channels, and binds with CaM to form a calcium/CaM complex (Chin and Means 2000;

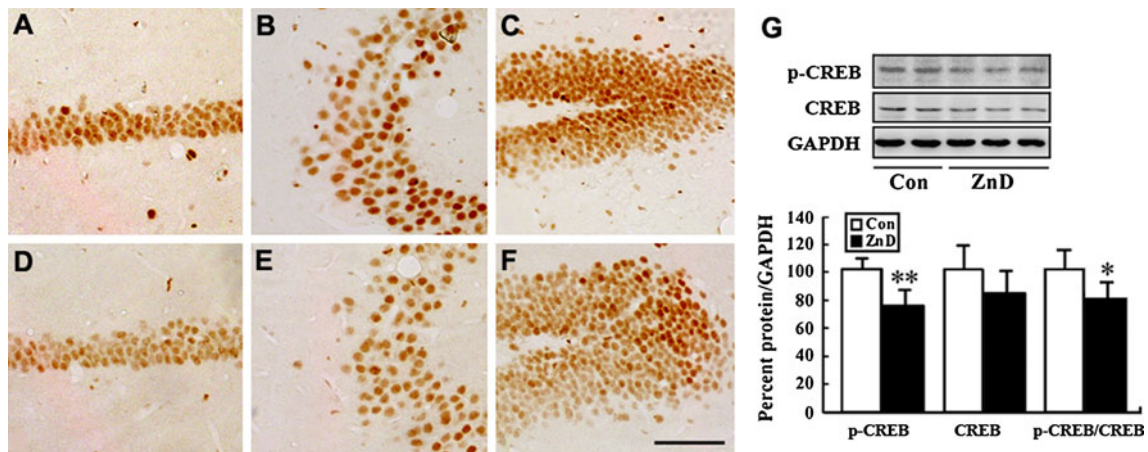


Fig. 4 Altered expression of p-CREB protein in the zinc-deficient mouse hippocampus. **a–f** Immunohistochemical images showing the distribution of p-CREB immunoreactivity in hippocampal CA1 (**a, d**), CA3 (**b, e**), and dentate gyrus (**c, f**) of controls (**a–c**) and zinc-deficient mice (**d–f**). p-CREB immunoreactivity was markedly reduced in the hippocampus of zinc-deficient mice. Scale bar = 50 μ m. **g** Representative immunoblot images showing the expression level of p-CREB and

CREB, respectively. GAPDH was used as a loading control. In the zinc-deficient hippocampus, a significantly reduced expression level of p-CREB protein was detected. However, no obvious difference in total CREB was found between the zinc-deficient (ZnD) and the control (Con) groups. The ratio of p-CREB/CREB was reduced in the zinc-deficient (ZnD) group when compared with the control (Con). * $P < 0.05$, ** $P < 0.01$; $n = 7$

Hudmon and Schulman 2002b; Miyamoto 2006). The activated calcium/CaM complex combines with CaMKII, resulting in its auto-phosphorylation on threonine residue 286 (Fink and Meyer 2002; Hudmon and Schulman 2002b). CaMKII activity and Thr286 autophosphorylation are essential for normal NMDA receptor-dependent forms of LTP and spatial learning and memory (Fink and Meyer 2002; Lisman et al. 2002; Matynia et al. 2002), and also for plasticity in other regions in the brain (Fang et al. 2002; Garry et al. 2003; Hardingham et al. 2003). Interestingly, zinc can directly activate CaMKII independently of calcium and CaM, and zinc can also affect CaMKII activity through modulating calcium/CaM binding to CaMKII (Lengyel et al. 2000). Our data showed that chronic zinc deficiency reduced the expression levels of CaM and p-CaMKII. The significantly reduced basal level of CaM probably directly affects the activated calcium/CaM complex itself, which results in retardation of CaMKII auto-phosphorylation without reducing its total level. Our data also showed a reduced basal level of Ng in the hippocampus of the zinc-deficient mouse, suggesting that the reduced level of p-CaMKII might also be attributed to the decline in the Ng level in the zinc-deficient hippocampus. Ng binds to CaM to produce a CaM/Ng complex. The reduced CaM/Ng complex can affect the activity of p-CaMKII. Collectively, down-regulation of Ng, CaM, and CaMKII phosphorylation by zinc deficiency might in part explain the impairment in learning and memory mechanism.

For CREB, several lines of evidence have confirmed the absolute requirement of this novel molecule for the growth

and the formation of new synaptic connections (Martin et al. 1997; Marie et al. 2005), and protein synthesis-dependent processes involved in the retrieval and reconsolidation of memory (Impey et al. 1996; Silva et al. 1998; Barco et al. 2002; Kida et al. 2002; Lonze and Ginty 2002). The significant upstream elements of the CREB pathway include calcium/CaM and CaMKII, which are essential for modulating the formation of phosphorylated CREB. Phosphorylation of CREB at Ser133 activates gene expression by binding CRE elements in promoter regions of several proteins and transcription factors that mediate neuronal plasticity and stimulate LTP generation (Mizuno et al. 2002). Consistent with a recent study showing that depletion of intracellular zinc down regulates the expression level of CREB mRNA in cultured hippocampal neurons (Liu et al. 2008), our in vivo experimental results demonstrated that dietary zinc deficiency markedly reduced the basal levels of p-CREB, the active form of CREB, but the level of total CREB was not changed in the hippocampus. The main reason for this would be the fact that the reduced level of p-CaMKII protein reduces CREB phosphorylation. In addition, we also examined whether dietary zinc deficiency could alter the expression level of CaN, another upstream molecule for the CaMKII/CREB signaling pathway that is involved in modulation of p-CaMKII activation. We did not detect a significant change in CaN level in the zinc-deficient hippocampus. These data suggest that zinc deficiency may selectively affect the expression of certain proteins, but not all, in the LTP-related CaMKII/CREB signaling pathway.

In summary, our findings indicate that severe dietary zinc deficiency hampers the learning and memory functions of the mouse hippocampus. This effect was associated with inactivation of the CaM/CaMKII/CREB signaling pathway in the hippocampus. We have recently reported that dietary zinc deficiency causes abnormal neurogenesis and neuronal apoptosis in the hippocampus (Xu et al. 2010; Gao et al. 2009), which is involved in abnormal neuronal plasticity and LTP induction. Taken together, these results suggest that multiple pathways related to learning and memory in the hippocampus could be affected by dietary zinc deficiency.

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