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ZINC PLUS CYCLO-(HIS-PRO) PROMOTES HIPPOCAMPAL NEUROGENESIS IN RATS

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Abstract—Zinc is a central actor in regulating stem cell proliferation and neurogenesis in the adult brain. High levels of vesicular zinc are found in the presynaptic terminals. It has been demonstrated that high levels of vesicular zinc are localized in the presynaptic terminals of the granule cells of the dentate gyrus (DG) and that neurogenesis occurs in the subgranular zone (SGZ). Furthermore, zinc chelation reduces hippocampal neurogenesis in pathological conditions such as hypoglycemia, epilepsy and traumatic brain injury. Here we test the effects of zinc plus cyclo-(His-Pro) (CHP) treatment on neurogenesis in the adult SGZ. In order to increase brain zinc, Sprague–Dawley (SD) rats, aged 5 weeks, were given zinc plus CHP (ZC, 27 mg/kg) orally available once per day for 2 weeks. BrdU was intraperitoneally injected 2 times per day for 4 consecutive days starting 1 week after initial ZC treatment. Neurogenesis was analyzed by BrdU, Ki67 and doublecortin (DCX) immunostaining. The number of progenitor cells and immature neurons were significantly increased in the DG following 2 weeks of ZC treatment. Hippocampal vesicular zinc content was evaluated with TSQ staining. Vesicular TSQ fluorescent intensity was seen to increase in the mossy fiber area at 2 weeks after ZC treatment. The present study demonstrates that zinc supplementation by ZC treatment increases hippocampal neurogenesis and levels of vesicular zinc. These findings provide evidence in support of the essential role of zinc in modulating hippocampal neurogenesis.

Key words: zinc, zinc plus cyclo-(His-Pro), neurogenesis, hippocampus.

INTRODUCTION

Adult neurogenesis occurs throughout the lifetime of mammals in restricted brain regions such as the subgranular zone (SGZ) of the dentate gyrus (DG) and the subventricular zone (SVZ). In the SGZ, proliferating cells eventually migrate into the granular cell layer (GCL) and functionally integrate into existing hippocampal circuitry (Kuhn et al., 1996; Eriksson et al., 1998; van Praag et al., 2002). Chelatable (loosely bound) zinc is found in high concentrations in the synaptic vesicles of mossy fiber terminals in the olfactory bulb and DG (Perez-Clausell and Danscher, 1985), both sites where neural migration and neurogenesis occur in the adult mammalian brain (Ming and Song, 2005). It is hypothesized that hippocampal network plasticity may be supported by these persistent changes (Toni et al., 2007, 2008; Vivar et al., 2012), although it remains unknown (Kempermann et al., 2004).

Histidine-proline-rich glycoproteins play a role in zinc transport (Borza and Morgan, 1998). Cyclo-(His-Pro) (CHP), a naturally occurring peptide, is enriched in the brain, as well as spinal cord, cerebrospinal fluid (CSF), blood, semen and gastrointestinal tract (Yanagisawa et al., 1980; Mori et al., 1983; Rosenthal et al., 2001; Song et al., 2001). CHP is also a neuromodulator, which is able to cross the blood–brain barrier (BBB) (Prasad et al., 1982; Banks et al., 1993), and zinc plus CHP (ZC) has been found to improve zinc absorption (Rosenthal et al., 2001), reduce blood glucose levels (Song et al., 1998, 2001; Hwang et al., 2003) and improve weight control (Song et al., 2009) in both type 1 and type 2 diabetics.

Zinc is stored in specialized vesicles localized to glutamatergic neurons (Bitanihirwe and Cunningham, 2009), and modulates brain excitability (Hambidge and Krebs, 2007), and influences learning and synaptic plasticity (Nakashima and Dyck, 2009; Tamano et al., 2015). Zinc also plays a role in the function of many enzymes, influences gene expression through transcription factors (Wu and Wu, 1987) and affects cell division, specifically in cells, which can be modulated by insulin-like growth factor-1 (IGF-1) (MacDonald, 2000) or nerve growth factor (NGF) (Stewart et al., 1984). Severe zinc deficiency also reduces division and migration of cerebellar granular cells (Dvergsten et al., 1983; Sandstead et al., 2000), both processes that are important to neurogenesis.

Vesicular zinc concentrations in hippocampal neurons are reduced in rats fed a zinc-deficient diet for 4 weeks and decreased dietary zinc correlates with a decline in

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Abbreviations: CSF, cerebrospinal fluid; DCX, doublecortin; DG, dentate gyrus; GCL, granular cell layer; GFAP, glial fibrillary acidic protein; NPCs, neural progenitor cells; NSCs, neural stem cells; SGZ, subgranular zone.

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learning (Golub et al., 1994; Takeda et al., 2000) and zinc supplementation improves spatial learning and memory (Tahmasebi Boroujeni et al., 2009; Karami et al., 2013; Piechal et al., 2016). Therefore, it is possible that dietary zinc availability may impact on neurogenesis.

In this study, we explored whether ZC administration can increase hippocampal neurogenesis under basal conditions. We found that ZC increased progenitor cell proliferation and immature neuron production. Furthermore, we found that ZC increased vesicular zinc in the hippocampus. These results together demonstrate that increasing hippocampal vesicular zinc by ZC promotes hippocampal neurogenesis and suggest that vesicular zinc plays a crucial role in the adult hippocampal neurogenesis.

EXPERIMENTAL PROCEDURES

Animals and ethics statement

Sprague–Dawley male rats (100–150 g, DBL Co, Chungcheongbuk, Korea), aged 4 weeks, were kept 2–3 per cage and housed under a 12-hour light/dark cycle with food and water *ad libitum*. To avoid stress associated with transportation, experiments were initiated after animals have been allowed a 1-week period of acclimatization to environmental conditions. Animal studies and experimental procedures were designed to minimize suffering and approved by the Committee on Animal Use for Research and Education at Hallym University (Protocol # Hallym 2014-30), according to NIH guidelines. This manuscript was also

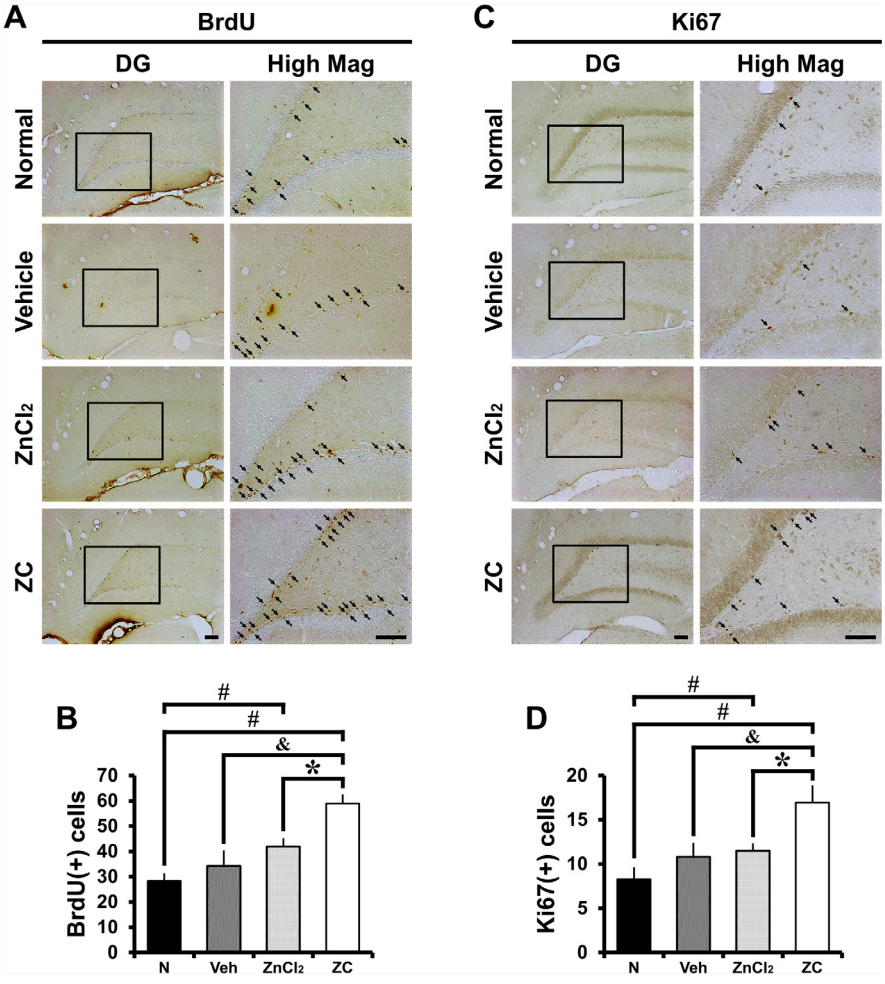

Fig. 1. ZC increases the proliferation of progenitor cells in the DG. Progenitor cell proliferation occurred in the dentate gyrus of rats. (A) Representative photomicrograph shows bromodeoxyuridine bound cells in the DG of rats. Brains were harvested at 2 weeks after initial ZC treatment and coronal sections were immunohistochemically stained for BrdU. BrdU-positive cells (arrows) were significantly increased in ZC-treated rats compared to normal, vehicle or $ZnCl_2$-treated rats. Scale bar = 100 μm. (B) Graph displays the number of BrdU-positive cells in the SGZ of DG. Data represent mean ± SE, n = 8–14 from each group. Normal vs. ZC, $^{\#}P < 0.05$; Vehicle vs. ZC, $^{*}P < 0.05$. (C) Representative photomicrograph of Ki67 immunostaining in the DG. Total Ki67-positive cells (arrows) were significantly increased in ZC-treated rats compared to normal, vehicle or $ZnCl_2$-treated rats. Scale bar = 100 μm. (D) Graph shows the number of Ki67-positive cells in the SGZ of DG. Data are expressed as mean ± SE, n = 8–14 in each group. $^{\#}P < 0.05$ compared with the normal group, $^{\&}P < 0.05$ compared with the vehicle group, $^{*}P < 0.05$ compared with the $ZnCl_2$ group.