ELSEVIER

Brain Research Bulletin 74 (2007) 278–283

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

Research report

Imunoreactivity of zinc transporter 7 (ZNT7) in mouse dorsal root ganglia

Li Zhang [a,b], Zhi-Hong Chi [a], Hao Ren [a], Ming Rong [a], Annica Dahlstrom [c], Liping Huang [d], Zhan-You Wang [a,*]

[a] *Department of Histology and Embryology, China Medical University, Shenyang 110001, PR China*
[b] *Department of Histology and Embryology, Jinzhou Medical University, Jinzhou 121000, PR China*
[c] *Department of Biomedicine, Sahlgrens Academy, Goteborg University, Goteborg, Sweden*
[d] *Department of Nutrition and Rowe Program in Genetics, USDA/ARS/Western Human Nutrition Research Center, University of California at Davis, Davis, CA, USA*

Received 21 June 2007; received in revised form 28 June 2007; accepted 29 June 2007
Available online 23 July 2007

Abstract

In the present study, we showed for the first time the localization of ZNT7 immunoreactivity in the mouse dorsal root ganglion (DRG) by means of immunohistochemistry and confocal laser scanning microscopy. Our results revealed that ZNT7 immunoreactivity was abundantly expressed in the nerve cells of the mouse DRG. Strong ZNT7 immunoreactivity was predominantly distributed in the perinuclear region of positive cells, while the nuclei were devoid of staining. Double immunofluorescence labeling of ZNT7 and TGN38 revealed a colocalization of the two antigens in the Golgi apparatus. In addition, the presence of labile zinc ions was detected with in vivo zinc selenium autometallography (AMG). AMG observations showed that the zinc staining pattern was also predominately located in the perinuclear Golgi area, like the ZNT7 immunostaining pattern in the DRG. These observations strongly suggest that ZNT7 may play an important role in facilitating zinc transport into the Golgi apparatus from the cytosol in the mouse DRG.

Keywords: Zinc; Zinc transporter; Golgi apparatus; Dorsal root ganglion; Immunohistochemistry; Autometallography

1. Introduction

Zinc, a divalent cation, is essential for development, growth, gene regulation, protein synthesis, intracellular protein trafficking, hormone function, immune function and a wide array of cellular processes [7,50,17,46]. In the brain, zinc is coreleased with neurotransmitters, and the interaction of zinc and neurotransmitters release may be involved in several neurobiological functions [36,48]. Zinc is also associated with aging and neurodegenerative disorders, such as Alzheimer's disease [45,1], Parkinson's disease [2], transient forebrain ischemia [15], seizures [16,30,12], and traumatic brain injury [27]. In the central nervous system (CNS), approximately 90% of total zinc is tightly bound to macromolecules to maintain the three-dimensional structure of a wide variety of proteins or to serve as a cofactor of a large number of enzymes [22–24]. The remaining 10% of total zinc, the chelatable zinc, is free or weakly bound to the host molecules and can be detected by zinc specific fluorescent dyes, such as zinquin and 6-methoxy 8-para toluene sulfonamide quinoline (TSQ) [52,25,26,11], and histochemically detectable with Timm staining or autometallography (AMG) [32,19,20,34,31]. The later is excellent for detecting nM levels of chelatable zinc ions in tissues.

In mammalian cells, intracellular zinc homeostasis is achieved through various mechanisms including zinc sensing, binding, and sequestering. Zinc sensing is mediated by regulating the expression of zinc transporters (ZNTs), which are believed to take part in zinc trafficking across membranes in living cells [37,14]. Zinc transporters are mainly assigned to two metal-transporter families: cation diffusion facilitator (CDF) and ZIP (ZRT, IRT-like protein) families [44,43,29]. The key feature of CDF-family transporters is that they transport zinc ions from the cytoplasm into the lumen of intracellular organelles

* Corresponding author. Tel.: +86 24 23256666 5305; fax: +86 24 23256666 5305.
E-mail address: wangzy@mail.cmu.edu.cn (Z.-Y. Wang).

0361-9230/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2007.06.022

or to the outside of the cell, while ZIP family transporters function in facilitating zinc trafficking in the opposite direction [39,3,10]. Most of the CDF family members are predicted to have similar topology with six transmembrane domains and a large histidine-rich intracellular loop between domains IV and V, which is supposed to function as a potential zinc binding site [43,29,3,10,59,28,9]. To date, eight members of the ZNT family, named ZNT1 to ZNT8, have been cloned and functionally characterized [8]. Although ZNT1-8 proteins posses high degree of sequence and structure homologies, they reside in different subcellular compartments, suggesting that they may play different roles in zinc homeostasis [14,28,35,47,40,41,42,33,57,38]. ZNT1 is responsible for transporting zinc from the cytosol to extracellular spaces, while the others, ZNT2-8, are involved in pumping zinc into different intracellular organelles [44]. All of these plasma membrane zinc efflux transporters play an important role in preventing the cellular zinc over accumulation thereby saving the cell from the toxic consequences of zinc overload [44,39,13].

Recently, it has been reported that ZNT7 exists in a variety of organs such as brain, retina, placenta, lung and gastrointestinal tract [35,6,5,58,54]. Immunofluorescence and immunohistochemical results reveal that ZNT7 resides in the Golgi apparatus and is responsible for transporting cytoplasmic zinc ions into the Golgi apparatus [35,6]. In the DRG, nevertheless, very little is known about the localization of ZNT7 and its possible role in zinc metabolism. In order to gain further insight on whether ZNT7 is expressed and is involved in zinc homeostasis in the DRG, we explored, accordingly, the localization of ZNT7 and chelatable zinc ions in the mouse DRG in the present study.

2. Materials and methods

2.1. Experimental animals

Male CD-1 mice (30–35 g, 8–10 weeks old) were used as experimental animals in the present study. They were housed under a 12 h light/dark cycle with water and food available ad libitum. All procedures were carried out in accordance with the ethical standards of China Medical University.

2.2. ZNT 7 immunohistochemistry

During deep anesthesia by sodium pentobarbital (50 mg/kg, i.p.), mice were perfused transcardially with isotonic saline, followed by a fixative of cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The L1-5 DRGs of both sides were carefully removed and further post-fixed in the same fixative for 3 h at 4 °C, and then transferred to 30% sucrose in PBS for cryoprotection at 4 °C overnight. The DRGs were embedded in a Tissue Tek OCT block and cut on a cryostat at a thickness of 10 μm and mounted on glass slides. An affinity-purified rabbit anti-ZNT7 polyclonal antibody was used for immunohistochemical detection of the ZNT7 protein in the DRG sections. The ZnT7 antibody was raised against a synthetic peptide from amino acids 299–315 of mouse ZNT7 (TPPSLENTLPQCYQRVQ), and the specificity was confirmed in our previous study [35]. The immunostaining procedures were performed in accordance with the standard ABC method. Briefly, sections were rinsed in 0.1 M Tris-buffered saline (TBS, pH 7.4), and endogenous peroxidases were quenched with 3% hydrogen peroxide (H_2O_2) in pure methanol for 10 min. After three washes with TBS, sections were treated with 5% bovine serum albumin (BSA) and 3% goat serum in TBS for 1 h to reduce nonspecific staining. Sections were then rinsed in TBS for 30 min and incubated overnight at 4 °C in ZNT7 antiserum, diluted 1:100 in TBS plus 3% goat serum and 1% BSA and 0.3% Triton-X 100. Following several rinses, sections were incubated in biotinylated goat anti-rabbit IgG at 1:200 for 1 h at room temperature (RT). They were rinsed and then incubated in ABC solution at RT for 1 h. A brown color was developed by rinsing sections in 0.1 M Tris buffer (pH 7.6) and then incubating sections in 0.025% 3,3′-diaminobenzidine (DAB) in the presence of 0.033% H_2O_2 at RT for 10 min. The sections were dehydrated and coverslipped for light microscopy.

As a negative control, some DRG sections were incubated without primary antibody and processed as described above. No distinct staining was observed.

2.3. Double labeling of ZNT7 and TGN38 immunofluorescence and confocal laser scanning microscopy

Double labeling of ZNT7 and trans-Golgi network 38 (TGN38; a Golgi marker) immunofluorescence was carried out to examine whether ZNT7 was colocalized with TGN38 in the Golgi apparatus. Cryostat sections of mouse DRG were prepared as above. They were preincubated with normal donkey serum (1:20) for 1 h, then incubated overnight with anti-ZNT7 (1:100; polyclonal antibody) and anti-TGN38 (1:100; monoclonal antibody, Sigma) at RT. After rinsing with PB, the sections were incubated with fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG (1:50) and Texas Red-conjugated donkey anti-mouse IgG (1:50) for 2 h at RT. The sections were rinsed with PB, mounted with an anti-fading medium, and examined with a confocal laser scanning microscope.

2.4. Zinc selenide autometallography

Zinc selenide autometallography (AMG) was used to analyze the distribution of chelatable zinc ions in the mouse DRG. Mice were injected intraperitoneally with sodium selenite (25 mg/kg) and allowed to survive for 1.5 h, after which they were deeply anesthetized with pentobarbital (50 mg/kg) and transcardially perfused with saline, followed by 2.5% glutaraldehyde in 0.1 M PB. The DRGs were carefully removed and immediately placed in the same fixative at 4 °C for 3 h. Samples were rinsed in PB and immersed in 30% sucrose overnight at 4 °C for cryoprotection, and further cut in 10 μm coronal sections on a cryostat, placed on Farmer rinsed slide glasses, and dipped in a 0.5% gelatin solution. Sections were incubated in the AMG developer in a 26 °C water bath for 1 h as described by Danscher and co-workers [11,53], followed by immersion in the AMG stop bath, 5% thiosulphate solution. The sections were placed under running tap water for 20 min in order to remove the gelatine membrane, and further dehydrated and coverslipped for light microscopy.

3. Results

3.1. Expression of ZNT7-immunoreactivity in the mouse DRG

Routine immunohistochemical observations revealed that almost all DRG neurons of different sizes were ZNT7-immunopositive. Fig. 1a shows the overall immunohistochemical localization of ZNT7 immunoreactivity in the mouse DRG. In general, a distinct ZNT7 immunostaining was observed throughout the cytoplasm of DRG neurons, while the nuclei were devoid of staining. At a higher magnification (Fig. 1b), the ZNT7 immunostaining was located predominantly in the perinuclear regions of the neurons, suggesting a Golgi apparatus localization of ZNT7 protein at light microscopic levels.

3.2. Colocalization of ZNT7 and TGN38 in the mouse DRG

To further test whether ZNT7 is located in Golgi apparatus of the mouse DRG neurons, double immunofluorescence labeling of ZNT7 and TGN38 was performed and examined in a confocal microscope. Confocal microscopic examination showed that