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BRAIN RESEARCH

Research Report

Zip6 (LIV-1) regulates zinc uptake in neuroblastoma cells under resting but not depolarizing conditions

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ARTICLE INFO

Article history:
Accepted 1 January 2008
Available online 18 January 2008

Keywords:
Zinc transporter
Brain
LIV-1
ZIP6
SLC39A6
Zinc uptake
Zinc influx

ABSTRACT

Impaired zinc homeostasis is implicated in many cases of brain injury and pathogenesis. While several routes of zinc influx have been identified in neurons under depolarizing conditions, zinc uptake mechanisms during resting conditions are unknown. We have previously detected Zip6 at the plasma membrane of rat neurons, suggesting a role for Zip6 in neuronal zinc uptake. Zinc uptake under resting and depolarizing membrane potentials was measured in SH-SY5Y neuroblastoma cells using ^{65}Zn. Zinc uptake was higher under depolarizing conditions, compared with resting conditions, and could be reduced by high extracellular calcium, gadolinium, or nimodipine, which suggests that L-type calcium channels are significant routes of zinc uptake under depolarizing membrane potential. In contrast, zinc uptake under resting conditions was not affected by calcium or calcium channel antagonists. Zip6 was localized to the plasma membrane in SH-SY5Y cells, and siRNA-mediated down-regulation of Zip6 expression reduced zinc uptake during resting, but not depolarizing conditions. Zinc treatment (100 μM Zn) reduced zinc uptake under resting, but not depolarizing conditions, which was associated with lower plasma membrane-associated and total Zip6 protein abundance. These results demonstrate that Zip6 functions as a zinc import protein in neuroblastoma cells, that zinc influx during resting and depolarizing conditions occurs via distinctly different processes in these cells, and suggest that neuronal zinc uptake may be down-regulated by excess zinc levels, but only under resting conditions.

1. Introduction

While little is known regarding the regulation of zinc homeostasis in the central nervous system, regulatory impairment is implicated in the pathogenesis of various conditions, including seizures, transient cerebral ischemia, blunt head trauma, glucose deprivation, and Alzheimer's disease (Frederickson et al., 2005). Many of these acute brain injury conditions are accompanied by extended neuronal depolarization (Bittigau and Ikonomidou 1997; Obrenovitch and Urenjak 1997; Dirnagl et al., 1999; Doble 1999) that can last for more than 1 h after insult (Sombati et al., 1991; Tanaka et al., 1997) and is associated with enhanced cell death. This has been postulated to result from excessive potassium influx (Weiss et al., 1993). Alternatively, excessive zinc may be imported from the presynaptic cleft as less neuronal death in the CA3 hippocampal region caused by kainate-induced seizures occurs in ZnT-3 null mice which lack synaptic zinc pools (Lee et al., 2000).

Several routes of zinc entry into neurons have been identified, including calcium permeable AMPA receptors (AMPARs),

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Abbreviations: Gd, gadolinium; MT, metallothionein; VGCC, voltage-gated calcium channel; ZIP, ZRT, IRT-like Protein; Zn, zinc

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doi:10.1016/j.brainres.2008.01.015

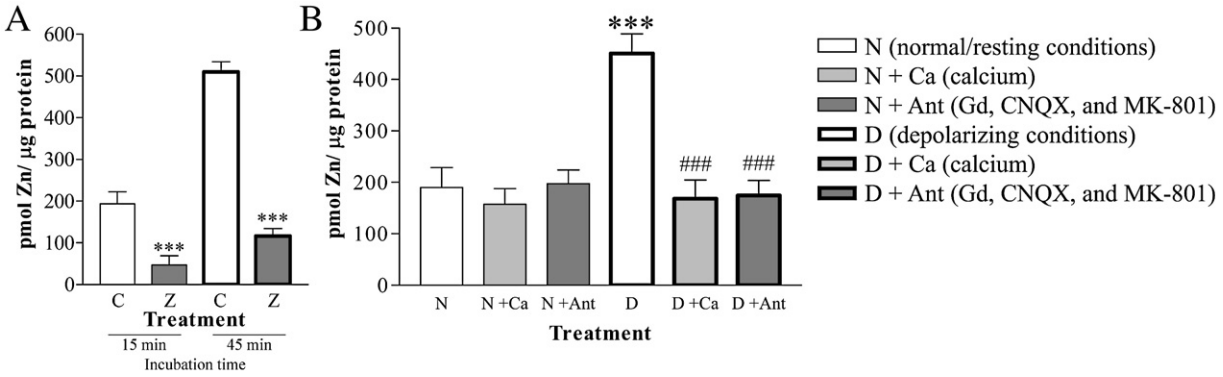

Fig. 1 – Distinct mechanisms of zinc uptake and regulation by zinc in neuroblastoma cells under resting and depolarizing conditions. (A) Zinc exposure reduced zinc uptake in neuroblastoma cells under resting conditions. Cells were incubated in control medium or medium supplemented with 100 µM Zn for 16 h. Zinc uptake was measured over 15 and 45 min under resting conditions. Data represented pmol Zn per µg protein per well (mean ± SD, *n* = 4). Means with asterisks differed from untreated cells at corresponding time point. (B) Separate mechanisms of zinc uptake in neuroblastoma cells under resting and depolarizing conditions. Zinc uptake was measured over 15 min. Membrane potential during zinc uptake measurement was either resting (N, normal) or depolarizing (D). Depolarizing conditions were created by an equimolar substitution of potassium for sodium during zinc uptake measurement. Zinc uptake was also measured in the presence or absence of extracellular calcium concentration (+Ca) or three calcium channel antagonists in combination: gadolinium, CNQX, and MK-801 (+Ant). Data represented pmol Zn per µg protein (mean ± SD, *n* = 4). Means with asterisks differed from untreated cells, normal resting potential. Means with pound signs differed from untreated cells, depolarized resting potential.

NMDA receptors (NMDARs), and voltage-gated calcium channels (VGCCs) (Sensi et al., 1997). While each of these routes is ligand- or depolarization-dependent, it has been suggested that there are other routes of zinc entry under resting conditions as cultured neurons under non-depolarizing conditions are also able to accumulate zinc (Colvin et al., 2000). Members of the SLC39A ion transporter gene family encode proteins (Zip1-14) which are involved in zinc influx (Eide 2004), and many have been shown to be regulated by zinc, either through alterations in mRNA expression, protein abundance, or cellular localization (Dufner-Beattie et al., 2003; Kim et al., 2004; Wang et al., 2004; Huang et al., 2005); however, there is currently little information regarding the expression or function of Zip proteins in the brain.

We have previously detected Zip6 (LIV-1) at the plasma membrane of rat neurons *in vivo* using immunohistochemistry (Chowanadisai et al., 2005), suggesting that Zip6 may mediate zinc influx into neurons. In this report, we investigated the function of Zip6 further using a human neuroblastoma cell model (SH-SY5Y). We determined that these cells express Zip6 and demonstrated that Zip6 is responsible for zinc influx in these cells. We report that zinc uptake in these cells is mediated by both depolarization-dependent and depolarization-independent

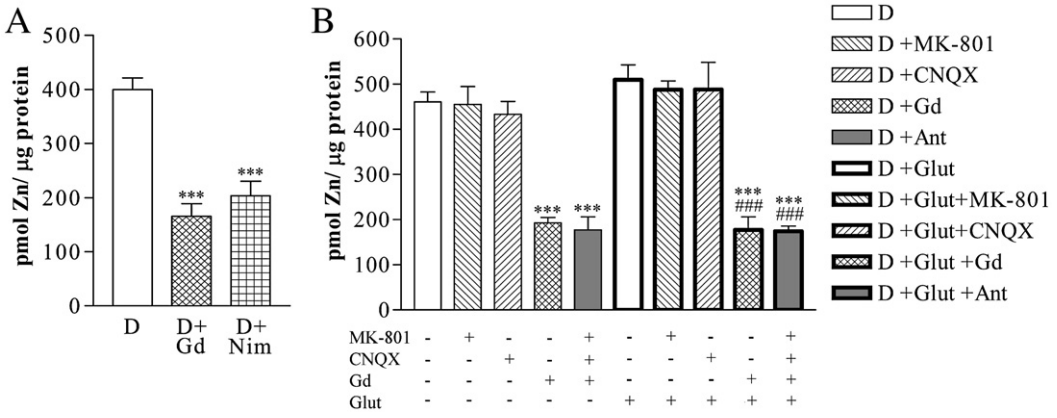

Fig. 2 – Zinc uptake during depolarization occurred primarily through L-type calcium channels. Zinc uptake was measured over 15 min. (A) Zinc uptake was measured in neuroblastoma cells under depolarizing conditions while exposed to either gadolinium (+Gd) or nimodipine (+Nim), in the absence of glutamate and glycine. Data represented pmol Zn per µg protein (mean ± SD, *n* = 4). Means with asterisks differed from untreated cells under depolarizing conditions. (B) Zinc uptake was measured in neuroblastoma cells under depolarizing conditions while exposed to a combination of calcium channel antagonists and/or glutamate and glycine. Calcium channel antagonists included MK-801, CNQX, gadolinium (Gd) and glutamate/glycine (Glut). Data represented pmol Zn per µg protein (mean ± SD, *n* = 4). Means with asterisks differed from untreated cells (D), and means with pound signs differed from cells treated with glutamate/glycine (D+Glut).