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Protective roles of selenium and zinc against postnatal protein-undernutrition-induced alterations in Ca^{2+}-homeostasis leading to cognitive deficits in Wistar rats

Olusegun L. Adebayo [a,b,c], Rajat Sandhir [b], Gbenga A. Adenuga [c,*]

[a] Department of Chemical Sciences, College of Natural Sciences, Redeemer's University, P.M.B. 230, Ede, Osun State, Nigeria
[b] Department of Biochemistry, Basic Medical Science Building, Panjab University, Chandigarh 160014, India
[c] Department of Biochemistry, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, P.M.B. 2005, Remo Campus, Ikenne, Ogun State, Nigeria

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ABSTRACT

Postnatal protein-undernutrition impacts on mental development and cognition in children and can lead to problem with attention and unresponsiveness which compromise children's ability to learn. These behavioral disorders might be due to alteration in calcium homeostasis as calcium plays critical roles in fundamental functions of neuron. The role of low protein diet as well as Se and Zn supplementation on intracellular calcium concentration ($[Ca^{2+}]_i$), Ca^{2+}-ATPase, Na^+-K^+-ATPase, calpain and caspase-3 activities from rat cortex and cerebellum were investigated. Well-fed (WF) and low protein diet-fed (LPDF) rats were given diets containing 16% and 5% casein, respectively, for a period of 10 weeks. Then, the rats were supplemented with Se and Zn at a concentration of 0.15 mg L^{-1} and 227 mg L^{-1}, respectively, in drinking water for 3 weeks. The results obtained from the study showed a significant increase in $[Ca^{2+}]_i$; calpain and caspase-3 activities as well as increase transfer latency in water maze study and reductions in Ca^{2+}-ATPase and Na^+-K^+-ATPase activities for LPDF rats compared to WF rats. Se and Zn supplementation to LPDF rats reversed the elevation in $[Ca^{2+}]_i$, calpain and caspase-3 activities and restored the cognitive deficits and the activities of Ca^{2+}-ATPase and Na^+-K^+-ATPase. Conclusively, protein-undernutrition results in the accumulation of synaptosomal calcium and inhibition of calcium transporters presumably via free radical generations and results in cognitive impairment which also probably results from neuronal death in rats through calpain activation and the caspase cascade mechanisms. However, Se and Zn supplementations ameliorated the anomalies observed.

1. Introduction

Protein undernutrition (PU) is a major problem in the tropic that causes structural and functional deficits leading to brain functions impairment (Waber et al., 2011). PU has been shown to affect several aspects of behavior and cognitive function, including learning and memory. Low dietary protein has been reported to cause severe micronutrients deficiency (Rohde et al., 2014) as well as induction of oxidative stress. Oxidative stress has been associated with many neurodegenerative diseases and other cellular damages. However, there are increasing evidence that antioxidants supplementation can fight and inhibit oxidative damage in the brain (Atif et al., 2008; Song et al., 2014).

Selenium and zinc are essential trace elements with antioxidant properties that are important in maintaining optimal brain functions (Wirth et al., 2010). Selenium is an important component of several enzymes like thioredoxin reductase, glutathione peroxidase (GP*x*), selenoprotein P and iodothyronine 5′-diodinase that is required in regulating gene expression, antioxidant system, and proliferation (Atif et al., 2008; Deepmala et al., 2013). Selenium has been shown to be neuroprotective against trauma and epilepsy (Song et al., 2014), acute ischemia and Alzheimer's disease (Ishrat et al., 2009). Zinc has long been recognized biologically for brain function and it is localized in the synaptic vesicle of mossy fiber of the dentate granule cell; which are sites where neurogenesis and neural migration are most active in the adult brain (Choi et al., 2014). Zinc protects against malnutrition-induced brain developmental impairments and it is also important in depression therapy and normal cognitive functioning (Ladd et al., 2010).

Calcium is a major signal transducer in living cells modulating many physiological and pathological functions such as neuronal

* Corresponding author. Tel.: +234 805 513 6857.
E-mail addresses: gadenuga@hotmail.com, gadenuga58@gmail.com (G.A. Adenuga).

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excitability, neurotransmitter synthesis and release, and many aspects of neuronal activity ranging from rapid modulation to changes in gene expression. It participates in long term processes like memory and learning, cell survival and synaptic transmission and plasticity (Kamboj and Sandhir, 2007). It causes cell death as overloads in local intracellular distribution determine toxicity or cell death (Berridge et al., 2003). When properly controlled, Ca^{2+} fluxes across the plasma membrane and between intracellular compartments where it plays critical roles in fundamental functions of neurons (Mattson 2007). However, deregulation of calcium homeostasis leads to neurodegeneration via complex and diverse mechanisms involved in selective neuronal impairments and death (Mattson 2007).

Implication of calcium in many neurodegenerative diseases leading to neurological disorders and cognitive deficits has been documented. However, there is paucity of information on the effect of low protein (LP) diet on calcium homeostasis in the brain. Thus, the present study attempted to evaluate the effect of postnatal PU on calcium homeostasis in rat brain and the effect of selenium and zinc supplementation (if any) on the parameter.

2. Materials and methods

2.1. Animals and diets

Male weanling Wistar rats were obtained from the Central Animal House of the Panjab University, Chandigarh, India. The rats were acclimatized for five days and were housed in polypropylene cages, fed with standard rat chow and water during this period before they were randomly grouped and fed with specially prepared diets. The LP diet contained 5% casein while the control diet contained 16% casein as previously reported by Adebayo et al. (2014). The rats were maintained at 12 h light dark-cycles at room temperature. All procedures on rats handling used in this study were approved by the Institutional Ethics Committee and were in accordance with the NIH Guidelines for Humane Use and Care of Laboratory Animals.

2.2. Experimental protocols

Rats were randomly assigned to six (6) groups. The control groups (well-fed; WF groups) were provided with diet containing 16% casein had six (6) rats per group while the low protein (LP) groups had eight (8) rats per group and were provided with diet containing 5% casein. Grouping of rats and treatments with Se or Zn at the end of 10th week were as follows:

Group A – Well fed (16% casein)
Group B – Low protein (5% casein)
Group C – Well fed + $NaSeO_3$
Group D – Low protein + $NaSeO_3$
Group E – Well fed + $ZnSO_4{\cdot}7H_2O$
Group F – Low protein + $ZnSO_4{\cdot}7H_2O$

Se and Zn were supplemented at a concentration of 0.15 mg L^{-1} and 227 mg L^{-1} (Adebayo et al., 2014), respectively, in drinking water for 3 weeks. The rats were sacrificed at the end of 13th week under mild ether anesthesia by decapitation. Brains were dissected into regions (cortex and cerebellum), rinsed in ice cold isotonic saline, and stored at −80 °C until required for analyses.

2.3. Preparation of synaptosomal fractions

Crude synaptosomal fraction was prepared according to the method of Gray and Whittaker (1962) as modified by Meder et al. (1997).

2.4. Measurement of synaptosomal intracellular calcium levels

Calcium levels were measured in synaptosomal fractions prepared from the cerebral cortex and cerebellum using Fura-2-acetomethoxyl according to the method of Meder et al. (1997). The results were expressed as nanomoles of free calcium.

2.5. Measurement of synaptosomal Ca^{2+}-ATPase

The activity of synaptosomal Ca^{2+}-ATPase was measured in synaptosomal fractions prepared from the cerebral cortex and cerebellum according to the method of Sepulveda et al. (2007). The results were expressed as nanomole min^{-1} mg $protein^{-1}$.

2.6. Determination of synaptosomal Na^+-K^+-ATPase activity

Na^+-K^+-ATPase was assayed in the crude synaptosomal fraction according to the method of Quigley and Gotterer (1969). The results were expressed as nanomoles of ATP hydrolyzed min^{-1} mg $protein^{-1}$.

2.7. Proteolytic activity assay of calpain

The measurement of the activity of calpain, a protease, was done according to the method of McDonald et al. (2001). The results were expressed as picomoles min^{-1} mg $protein^{-1}$.

2.8. Fluorometric assay of caspase-3 activity

The fluorometric assay of caspase-3 was determined by the method of Bizat, et al. (2003). The activity of caspase-3 was expressed as AMC released min^{-1} mg $protein^{-1}$.

2.9. Estimation of inorganic phosphate

The inorganic phosphate was estimated according to the method of Stewart (1974) using disodium hydrogen as standard. The amount of inorganic phosphate released was expressed as picomoles P_i^{-1} min^{-1} mg $protein^{-1}$.

2.10. Estimation of protein

The protein content was determined according to the method of Lowry et al. (1951) using bovine serum as standard.

2.11. Morris water maze (MWM)

Morris water maze (MWM) is a device for investigating spatial learning and memory in the laboratory. The water maze was carried out according to Morris (1984).

3. Statistical analysis

Results were analyzed using one-way analysis of variance. Differences between means were determined by the use of Duncan multiple range test (SPSS software, version 17). Values with $p < 0.05$ were considered as statistically significant.

4. Results

4.1. Effect of low protein diet, Se and Zn supplementation on synaptosomal calcium level

The results showed (Fig. 1) that LP diet significantly ($p < 0.05$) increased intracellular calcium ion concentration $[Ca^{2+}]_i$ in the