ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 10, pp.59958-59963, October, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EFFECT OF MANGANESE ON ENERGY METABOLISM IN YOUNG AND ADULT RAT BRAIN: AMELIORATING EFFECT OF ALPHA-TOCOPHEROL

***,[1]Bhuvaneswari Devi Chinthirla and [2]Kiran Kumari Konduru**

[1]Department of Cell Biology and Neuroscience, University of California Riverside, CA, USA
[2]Department of Zoology, S.P.W. Degree and PG College, Affiliated to Sri Venkateswara University, Tirupati-517502, India

ARTICLE INFO

Article History:
Received 23rd July, 2017
Received in revised form
27th August, 2017
Accepted 10th September, 2017
Published online 31st October, 2017

Key words:

Brain Regions,
Manganese,
Alpha-Tocopherol,
Na^+K^+ATPase
and Mg^{2+}ATPase.

ABSTRACT

Trace amounts of Manganese (Mn) are essential for the proper functioning of a variety of physiological processes, including development, in all living organisms. However, several studies indicate that high Mn levels may be toxic to terrestrial and aquatic organisms, especially due to its neurotoxic properties. In the present study, the young and adult albino rats (2 months and 4 months old) were exposed to low dose (2.5mg/kg body weight) and high dose (5mg/kg body weight) of Mn through intraperitoneal injection for a period of 3 weeks and a separate batch treated with Mn was left for a period of one week supplementation with Alpha-tocopherol at a dose of 5mg/kg body weightalong with Mn until the end of the study.Control animals received only deionized water without Mn. In the present study, it was observed that, the potential effect of Adenosine Triphosphatase (ATPase) activity (EC 3.6.1.3); Mg^{2+}ATPase and Na^+K^+ ATPase activities were assayed. From our observation, it was cleared that the Mg^{2+}ATPase and Na^+K^+ ATPase activities were decreased in synaptosomal fraction at both 2 months and 4 months old rats at both concentrations (i.e. 2.5 mg/kg bw and 5 mg/kg bw) when compared to control rats. The adult rats showed greater frequency of Mg^{2+}ATPase activity compared to young rats. However, the high dose (5 mg/kg bw) of Mn treated rats of both age groups showed decreased Mg^{2+}ATPase activity compared to low dose (2.5 mg/kg bw) of Mn treated rats. Whereas, the rats supplemented with Alpha-tocopherol along with Mn showed gradual increase in Mg^{2+}ATPase and Na^+K^+ATPase activities respectively. In addition to this, we have also examined the body weights of 2 months and 4 months old rats at both low dose and high dose of Mn and Alpha-tocopherol also. In this, it was clear that, the low and high dose Mn exposed rats showed decrease in their body weights at both ages (2 and 4 months) as compared to control. Decrease in body weights were higher in high dose exposure compared to low dose. However, partial recovery of body weights were observed in Alpha-tocopherol Supplemented rats at both ages in high dose and low dose of Mn exposure. The above findings suggest that short-term Mn *in vivo* administration causes a statistically significant decrease in energy metabolism and body weights. The Mn toxicity was reversed with Alpha-tocopherol co-administration which could thus be considered for future applications as a neuroprotective agent against chronic exposure to Mn and the treatment of manganism.

Citation: Bhuvaneswari Devi Chinthirla and Kiran Kumari Konduru, 2017. "Effect of Manganese on energy metabolism in young and adult rat brain: Ameliorating effect of Alpha-Tocopherol", *International Journal of Current Research*, 9, (10), 59958-59963.

INTRODUCTION

Evidence is accumulating that, in chronic Mn toxicity, the central dopaminergic and other neurotransmitter systems may be disturbed (Cotzias *et al.,* 1971; Bonilla, 1978; Lai *et al.,* 1984). However, the effect of chronic Mn encephalopathy on other aspects of brain metabolism have not been so extensively investigated (Lai *et al.,* 1984). More recently, there is some morphological, biochemical, and behavioral evidence that the developing brain may be susceptible to Mn toxicity (Chandra *et al.,* 1979; Lai *et al.,* 1984). James *et al.,* (1991) previously demonstrated that, in vitro, Mn inhibits synaptosomal sodium-potassium-activated and magnesium-activated adenosine triphosphatase (Na-K-ATPase and Mg-ATPase) activities (Lai *et al.,* 1980) and a variety of synaptosomal uptake systems (Lai *et al.,* 1978, 1980, 1981b, 1982c, 1984; Wong *et al.,* 1980). It is generally accepted that cerebral metabolism of glucose, which appears one of the determinants of tissue ATP level, is crucial for central nervous system (CNS) activity. Na^+K^+ -stimulated ATPase (E.C. 3.6.1.3.) is known to be involved in the maintenance of sodium and potassium gradients across

Corresponding author:* **Bhuvaneswari Devi Chinthirla,
Department of Cell Biology and Neuroscience, University of California Riverside, CA, USA.

plasma membranes at the expense of ATP hydrolysis, with very high activity in electrically excitable tissues. The activity of this enzyme has been found to increase in the developing rat brain and to decrease during aging (de Sousa *et al.,* 1978). A study on the effect of aging on Na^+ K^+ ATPase activity in crude synaptosomal fractions from the rat brain parietal cortex, hippocampus and striatum revealed a progressive decline in enzyme activity from 12 months to 24 months of age (Kaur *et al.,* 1998). Zaidi and co-workers (1998) found that also Ca^{2+}-ATPase activity in F344/BFN1 rat brain synaptic plasma membranes exhibited a progressive age dependent decrease which might be accounted for some loss of PMCA from the membranes and for age-related structural changes of calmodulin. In view of the above, we carried out our research on heavy metal Mn by using low dose and high dose in 2 months and 4 months rats short term exposure might provide a suitable experimental basis for the evaluation of the possible primary targets of Mn neurotoxicity, and energy metabolism. The aim of this study was to shed more light on the effects of Mn administration on: two important adenosine triphosphatases, namely, Na^+ K^+ATPase (an enzyme implicated in neuronal excitability, metabolic energy production) and Mg^{2+}ATPase (an enzyme functioning in order to maintain high brain intracellular Mg^{2+}), and body weights of young and adult rats. Moreover, α-tocopherol is a well-known antioxidant and chelating agent, it was co-administered with Mn in order to evaluate its efficacy on protecting the rat brain against the Mn-induced toxic effects on the above parameters.

MATERIALS AND METHODS

Chemicals

Manganese and Vitamin-E was selected as test chemical. The chemicals used in this study namely sucrose buffer, tris buffer, Tris-HCl, EDTA, Magnesium chloride, potassium chloride and sodium chloridewere obtained from Sigma, USA. The remaining chemicals obtained from Qualigens, India.

Procurement and maintenance of experimental animals

Young albino rats (Wistar) were purchased from IISc, Bangalore and maintained in the animal house of Y.V. University. The animals were housed in clear plastic cages with hardwood bedding in a room maintained at $28^0 \pm 2^0$ C and relative humidity 60 ± 10% with a 12 hour light/day cycle. The animals were fed in the laboratory with standard pellet diet supplied by Sri Venkateswara Traders, Bangalore and water *adlibitum*. The protocol and animal use were approved by Institution Animal Ethical Committee, Y.V. University.

Animal exposure to Mn and Alpha-tocopherol (Vitamin – E)

The young albino rats (both 2 months and 4 months old) were exposed to a low dose of 2.5mg/kg body weight and a high dose of 5mg/kg body weight through intraperitoneal injection for a period of 3 weeks and a separate batch treated with Mn was left for a period of one week supplementation with Alpha-tocopherol at a dose of 5mg/kg body weight through intraperitoneal injection. After the period of dosage, the animals were sacrificed through cervical dislocation and the tissues were stored at -80^0C for the further biochemical analysis.

Biochemical Studies

Preparation of Crude Synaptosomal Fraction

Brain synaptosomes were prepared by homogenizing in 10 volumes (w/v) of 0.32 M sucrose buffer (0.32 M sucrose, 10 mM Tris-HCl, and 0.5 mM EDTA, pH 7.4).The homogenate was first Centrifuge at 1000*g* for 10 min at 4°C, and then the supernatant was centrifuged at 12,000*g* for 20 min. The buffy layer of pelleted synaptosomes was suspended in a low K_-HEPES buffer (125 mMNaCl, 5 mMKCl, 1.2 mM CaCl2, 1.2 mM Na2HPO4, 1.2 mM MgCl2, 5 mM NaHCO3, 10 mM HEPES, and 10mM glucose, pH 7.4).

Estimation of Adenosine Triphosphatase (ATPase) activity (EC 3.6.1.3)

Na^+K^+ and Mg^{2+}ATPase activities in the tissues were estimated following the method of Tirri *et al.* (1973). 1% homogenates of the tissues were prepared in 0.25 M ice cold sucrose solution. Homogenates were divided into two parts. One part was centrifuged at 1400g and the supernatant thus obtained was used as an enzyme source for Mg^{2+}ATPase, while the other part of the homogenate was used for the estimation of the total ATPase.

Mg^{2+}ATPase

The reaction mixture for Mg^{2+}ATPase assay contained 0.5 ml of tris buffer (0.13 M; pH 7.4), 0.4 ml of substrate ATP, 0.5 ml of Magnesium chloride (0.05 M $MgCl_2$) and 0.2 ml of crude homogenate/ mitochondrial fraction (enzyme source). The contents were incubated at 37° C for 15 minutes and the reaction was stopped by the addition of 10% TCA. Zero time controls were maintained by adding TCA prior to the addition of homogenate/mitochondrial fraction. The contents were centrifuged at 1000g for 15 minutes and the inorganic phosphate was estimated in the supernatant fraction following the method of Fiske and Subbarow (1925).

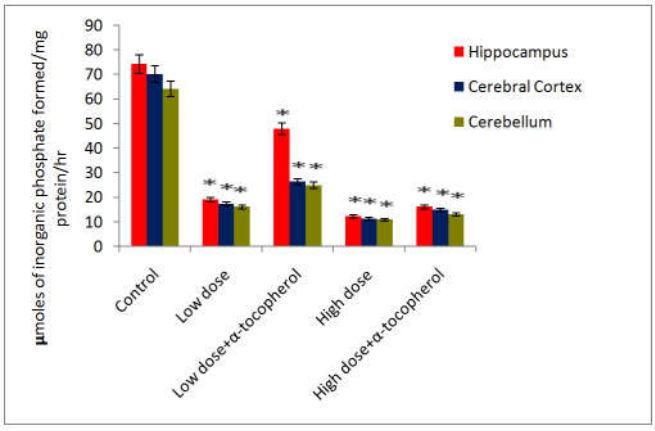

Fig.1. Alterations in Mg^{2+}ATPase activity (µ moles of inorganic phosphate formed/mg protein/hr) in synaptosomal fraction of young rats exposed to low dose (2.5 mg/kg bw) and high dose (5 mg/kg bw) of Mn and α-tocopherol (Vitamin E) administration. The values marked with asterisk (*) are significant from controls at p<0.05

Na^+K^+ATPase

1% (W/V) homogenate already set apart was used for the total ATPase assay. The reaction mixture in a final volume of 2.6 ml contained, 0.5 ml of Tris buffer (0.13 M; pH 7.4), 0.4 ml of

substrate ATP, 0.5 ml $MgCl_2$ (0.05 M), 0.5 ml potassium chloride (KCl, 0.05 M), 0.5 ml of sodium chloride (NaCl, 0.05 M) and 0.2 ml of crude homogenate/ mitochondrial fraction (enzyme source). The contents were incubated at 37° C for 15 minutes and the reaction was arrested by the addition of 1.5 ml of 10% TCA prior to the addition of homogenate. The contents were centrifuged and the inorganic phosphate was estimated in the supernatant fraction.

$Na^+ K^+$ATPase = Total ATPase - Mg^{2+}ATPase

Statistical treatment of the data

The mean and standard deviation (SD), analysis of variance (ANOVA) and test of significance or students 't' test was calculated using standard statistical software package.

RESULTS

Mg^{2+}ATPase activity

The results showed decreased Mg^{2+}ATPase activity in brain synaptosomal fraction at both 2 months and 4 months old rats at both concentrations (i.e. 2.5 mg/kg bw and 5 mg/kg bw) when compared to control rats. The adult rats showed greater frequency of Mg^{2+}ATPase activity compared to young rats. However, the high dose (5 mg/kg bw) of Mn treated rats of both age groups showed maximum decrease inMg^{2+}ATPase activity compared to low dose (2.5 mg/kg bw) of Mn treated rats. From the results, in the Fig.1, it was observed that the Mg^{2+} ATPase activity was significantly elevated in Mn treated young rat brain regions as compared to control. Further, simultaneous administration of alpha-tocopherol controlled Mn-induced Mg^{2+} ATPase activity respectively. The rats which were subjected to Mn at 4 months age, from Fig.2,it was clear that the total Mg^{2+}ATPase activity increased in adult rats (4 months) as compared to young rats. From our observations, the hippocampus showed maximum activity as compared to other brain regions.

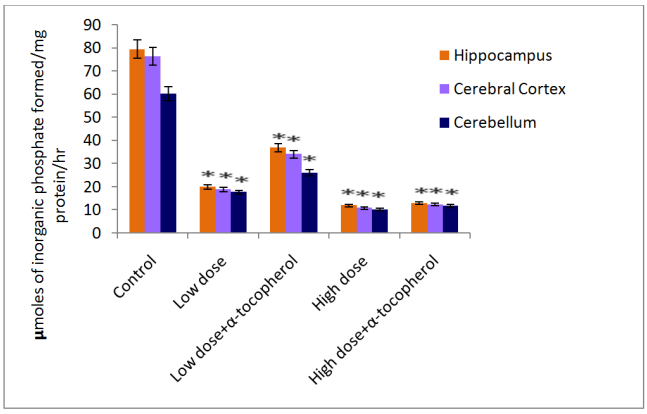

Fig.2. Alterations in Mg^{2+}ATPase activity (µ moles of inorganic phosphate formed/mg protein/hr) in synaptosomal fraction of adultrats exposed to low dose (2.5 mg/kg bw) and high dose (5 mg/kg bw) of Mn and α-tocopherol (Vitamin E) administration.The values marked with asterisk (*) are significant from controls at p<0.05

$Na^+ K^+$ ATPase activity

The results shown in Fig.3 and 4, decreased $Na^+ K^+$ ATPase activity in brain synaptosomal fraction at both 2 months and 4 months old rats was observed at both concentrations (i.e. 2.5 mg/kg bw and 5 mg/kg bw) when compared to control rats. The Mn-exposed rats showed a marked reduction in Na^+K^+ ATPase activity as compared to control. Alpha-tocopherol treatment along with Mn exposure showed maintenance of activity levels of Na^+K^+ ATPase in the control range. The adult rats showed greater frequency of Na^+ K^+ ATPase activity compared to young rats. However, the decrease was more in high dose (5 mg/kg bw) of Mn treated rats of both age groups compared to low dose (2.5 mg/kg bw) of Mn treated rats. Among the three brain regions studied cortex showed maximum activity followed by hippocampus and then cerebellum.

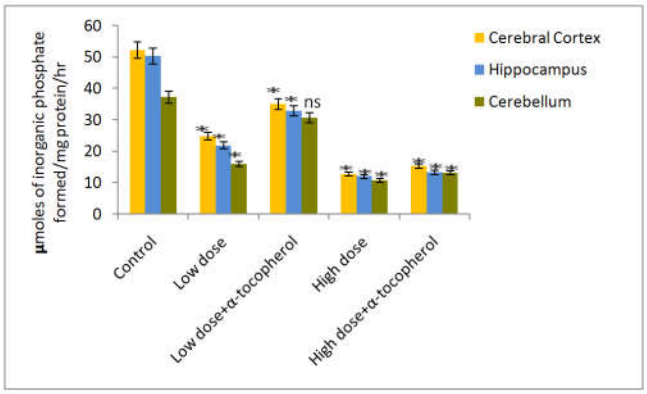

Fig.3. Alterations in Na^+K^+ATPase activity (µ moles of Pi formed/mg protein/hr) in synaptosomal fraction of young rats exposed to low dose (2.5 mg/kg bw) and high dose (5 mg/kg bw) of Mn and α-tocopherol (Vitamin E) administration.The values marked with asterisk (*) are significant and (ns) not significant from controls at p<0.05

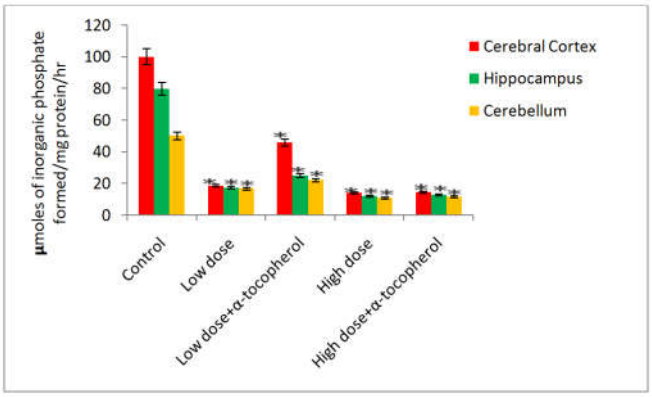

Fig.4. Alterations in Na^+K^+ATPase activity (µ moles of Pi formed/mg protein/hr) in synaptosomal fraction of adultrats exposed to low dose (2.5 mg/kg bw) and high dose (5 mg/kg bw) of Mn and α-tocopherol (Vitamin E) administration.The values marked with asterisk (*) are significant from controls at p<0.05

Body weights

From Fig.5, the low and high dose of Mn exposure rats showed decrease in their body weights in young rats (2 months) when compared with control rats. Decrease in body weights were higher in high dose exposure compared to low dose. However, partial recovery of body weights were observed in α-tocopherol supplemented rats along with Mn in low dose and high dose of Mn exposure. Similarly, from Fig.6, the adult rats treated with low dose and high dose of Mn showed loss in their body weights. The adult control rats showed increased body weights as compared to Mn treated rats. However, the recovery

of body weights were observed in α-tocopherol supplemented rats along with Mn in lowand high doses respectively.

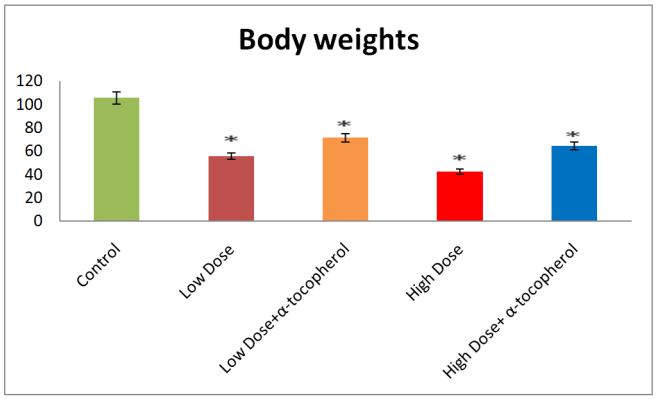

Fig.5. Body weights of 2 months old control, low and high dose of Mn exposed and Mn^{+} α-tocopherol supplemented rats. Each bar represents mean±SD (n=6). The values marked with asterisk (*) are significant from controls at p<0.05

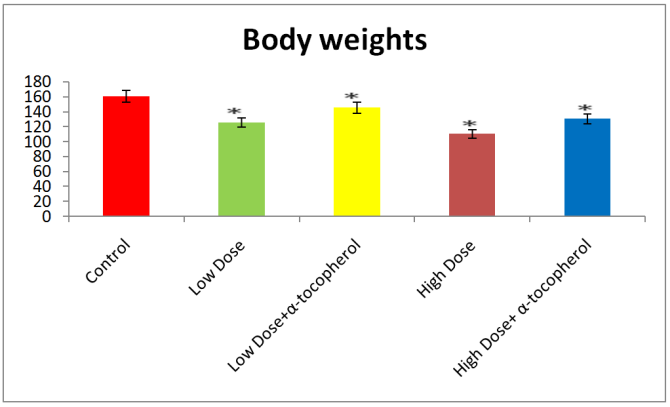

Fig.6. Body weights of 4 months old control, low and high dose of Mn exposed and Mn^{+} α-tocopherol supplemented rats. Each bar represents mean±SD (n=6). The values marked with asterisk (*) are significant from controls at p<0.05

DISCUSSION

Although Mn is an essential element, exposure to excessive levels of Mn can cause a variety of neurotoxic effects that involve (among others) alterations in cholinergic system and bioenergetics which finally leads to alterations in oxidative stress biomarkers (Villalobos and Suarez, 2001; Erikson and Dorman, 2007). Our data revealed a statistically significant Mn-induced reduction in $Na^{+}K^{+}$ATPase and Mg^{2+}ATPase activities in three brain regions (Cerebral cortex, cerebellum and hippocampus). When an antioxidant, Vit-E was administrated post Mn exposure, lead to the reversal in the alterations caused by the Mn both in terms of cholinergic and bioenergetics systems. And this could be due to the chelating properties of vit-E assisting to the biological inactivation and/or excretion of Mn ions. It should be noted that Mn brain concentrations have not been related to the extent of ACh content (Chen and Cheng, 2006) observed in certain animal brain regions after exposure to Mn, and that such alterations might be (in some extent) reversible (Erikson and Dorman, 2006). However, Vit-E (at least under the examined experimental conditions) was proved sufficiently efficient to neutralize the Mn-induced neurotoxicity. In this study, the activity of $Na^{+}K^{+}$ATPase was affected by Mn. Some studies (Atkinson and Hunt, 1968) have reported that Mn can inhibit $Na^{+}K^{+}$ATPase at high concentrations (>1 mM), but it can also activate $Na^{+}K^{+}$ATPase at lower concentrations. The studies of (Atkinson and Hunt, 1968) have shown that Mn^{2+} could replace the Mg^{2+} of the Mg^{2+}ATPase, at a certain Mn concentration. This chemical property of Mn might result in a (slight but significant) decrease of Mg^{2+}ATPase activity, a fact that was also observed in our experiments. Because Mg^{2+}ATPase is an enzyme functioning in order to maintain high brain intracellular Mg^{2+}, and thus possibly controlling the rate of protein synthesis and cell growth (Sanui and Rubin,1982), the high intracellular Mn^{2+} levels might be the reason for the observed inhibition. On the other hand, the co-administration of Vit-E reversed the Mn inhibition against Mg^{2+}ATPase. The concentrations used were below the levels which cause significant haemolysis (less than or equal to 300 mM). Mn^{2+} inhibited concentrative choline accumulation over a 3 h exposure period. The effects of Mn^{2+} on choline accumulation were reversed by removing the cations from the extracellular medium. Mn^{2+} also inhibited the efflux of choline. This inhibition of choline accumulation and efflux in erythrocytes by Mn^{2+} is not explicable solely in terms of either inhibition of Ca^{2+}–Mg^{2+}ATPase or inhibition of Na^{+}–K^{+}ATPase, which causes reduced intracellular K^{2+}. These findings are similar to those previously demonstrating the ubiquity (Finkelstein *et al.,* 2007) of manganese-induced effects on choline uptake in different cells and organ systems.
In another study, combined Pb+Mn-exposure exerted inhibitory action on the activity of enzymes Mg^{2+} and $Na^{+}K^{+}$ATPases in the developing brain. Heavy metals such as Pb can bind to a number of sites on proteins including imidazole, histodyl, carbonyl and especially sulfhydryl side chains (Kench, 1972).

Heavy metals have great affinity for ATPase system and they interact with enzyme molecule resulting in the inhibition (Walton and Gill, 1973). Pb has been reported to inhibit $Na^{+}K^{+}$ATPase of mammalian tissues (Cross *et al.,* 1970; Cardone *et al.,* 1971) and also interferes with mitochondrial function and blocks the O_2 uptake. Bhaumik and Raychudhari (1976) reported that the inhibition of $Na^{+}K^{+}$ATPase may be due to the flow of the Na^{+} K^{+} ions from the tissues to the blood. The decrease in the Mg^{2+}ATPase activity might be due to low operation of oxidative pathway, resulting in decreased formation of free energy and altered cellular energy metabolism (Boyer, 1977). The combination of Pb^{2+} and Mn^{2+} produced a pronounced decrease in the activity of Na^{+} K^{+}ATPase, but the magnitude of the change was the sum of the individual metal effects (Hussain *et al.,* 1987). It is known that brain derived $Na^{+}K^{+}$ATPase is among the enzymes particularly affected by Pb (Siegel *et al.,* 1977; Fox *et al.,* 1991). The decrease of $Na^{+}K^{+}$ATPase activity can change the gradients of Na^{+} and K^{+} across the cell membrane and can be the cause of the disturbances in neurotransmitters levels (Fox *et al.,* 1991). The observed high activity of ATPase in the cortex, cerebellum and hippocampus regions of the brain suggests the involvement of these regions in different behavioral functions. It is known that the level of ATPase parallels the metabolic demands of different regions of rat brain and the differential sensitivity to Pb+Mn neurotoxicity in these brain regions is not due to a preferential metal accumulation, but is possibly due to alteration of biochemical or cellular processes that are uniquely associated with, or greatly enhanced in a particular region (Widzowski and Cory-Slechta, 1994; Moreira *et al.,* 2001). Regional variations in AChE(Reddy *et al.,* 2007) and ATPases activity levels observed in different brain regions could be due

to structural and functional differences in brain regions. Thus, from the present study, it is evident that developmental Mn-exposure inhibited the Mg^{2+}ATPase and $Na^{+}K^{+}$ATPase enzymes in a dose dependent manner and α-tocopherol supplementation significantly reversed the Mn-induced alterations in Mg^{2+}ATPase and $Na^{+}K^{+}$ATPase activities.

Siomara C. Monteiro *et al.* 2007, investigated the influence of vitamins E plus C on the effects elicited by ovariectomy on the activities of these enzyme in hippocampus of ovariectomized rats. They demonstrated that vitamins E plus C reversed the activation of Na^{+}, K^{+}ATPase in hippocampus of ovariectomized rats. In this study, our findings were corroborate with Siomara C. Monteiro *et al.,* 2007, findings. In the present study, we have observed that the $Na^{+}K^{+}$ATPase and Mg^{2+} ATPase activities were decreased in Mn treated rats. Whereas, the rats supplemented with vitamin E along with Mn showed gradual decrease in $Na^{+}K^{+}$ATPase and Mg^{2+}ATPase activities respectively. In our study, low and high dose of Mn exposure showed alterations in body weights as compared to controls and slight increase was observed with Vitamin E supplemented rats. It was proved that Mn has bi-directional effect on the animals, both deficient and excess intake of Mn result in altered enzymatic reactions and brain function because of its essential element nutrition and neurotoxicant effect (Burton and Guilarte 2009).

Acknowledgement

The authors express their heartfelt thanks to Department of Science and Technology (DST) New Delhi for providing financial assistance for conducting the research work.

REFERENCES

Atkinson, A., Hunt, S., Lowe, A.G., 1968. Manganese activation of a (Na^{+},K^{+})-dependent ATPase in pig brain microsomes. *BiochimBiophys Acta*. 167: 469–72.

Bhaumik, Raychudhari., 1976. *Ind. J. Physiol. Allied. Sci.,* 29(4): 111.

Bonilla, E., 1978. Increased GABA content in caudate nucleus of rats after chronic manganese chloride administration. *J. Neurochem.,* 31: 551–552.

Boyer, P., 1977. In: The Enzymes. *Academic press, NY. 3rd Edn*. Vol. 13. *Brain Res*., 1258: 86–95.

Burton NC and Guilarte, TR 2009. Manganese Neurotoxicity: Lessons Learned from Longitudinal Studies in Nonhuman Primates. *Environmental Health Perspectives*, 117(3), 325-332.

Cardone, E., Lessler, M.A., Brierley, G.P., 1971. Mitochondrial oxidative phosphorylation interaction of lead and inorganic phosphate. *Proc. Soc. Exp. Biol. Med.,* 136: 300.

Chandra, S.V., Shukla, G.S., Saxena, D.K., 1979. Manganese-induced behavioral dysfunction and its neurochemical mechanism in growing mice. *J. Neurochem.,* 33: 1217–1221.

Chen, M.T., Cheng, G.W., Lin, C.C., Chen, B.H., Huang, Y.L., 2006. Effects of acute manganese chloride exposure on lipid peroxidation and alteration of trace metals in rat brain. *Biol Trace Elem Res*., 110: 163–78.

Cotzias, G.C., Papavasiliou, P.S., Ginos, J., Steck, A., Duby, S., 1971. Metabolic modification of Parkinson's disease and of chronic manganese poisoning. *Ann. Rev. Med.,* 22: 305–326.

Cross, R.L., Cross, B.A., Wang, J.H., 1970. Detection of a phosphorylated intermediate in mitochondrial oxidative phosphorylation. *Biochem. Biophys. Res. Commun.,* 10; 40(5): 1155-61.

Crossgrove, J., Zheng, W., 2004. Manganese toxicity upon exposure. *NMR in Biomedicine*, 17: 544-553.

De Sousa, B.N., Kendrick, Z.V., Roberts, J., Baskin, S.I., 1978. Na^{+},K^{+} -ATPase in brain and spinal cord during aging. *Adv Exp Med Biol.,* 97: 255 - 258.

Erikson, K.M., Dorman, D.C., Fitsanakis, V., Lash, L.H., Aschner, M., 2006. Alterations of oxidative stress biomarkers due to *in utero* and neonatal exposures of airborne manganese. *Biol Trace Elem Res.,* 111: 199–215.

Erikson, K.M., Dorman, D.C., Lash, L.H., 2007. Manganeses inhalation by Rhesus monkeys is associated with brain regional changes in biomarkers of neurotoxicity. *Toxicol Sci*., 97(2): 459-466.

Finkelstein, Y., Milatovic, D., Aschner, M., 2007. Modulation of cholinergic systems by manganese. *Neurotoxicology*., 28:1003– 14.

Fiske, C.H., Subba Row, Y., 1925. The colorimetric determination of phosphates. *J. Biol. Chem.* 66: 375-400.

Fox, D.A., Rubinstein, S.D., Hsu, P., 1991. Developmental lead exposure inhibits adult rat retinal, but not kidney, Na+ K+ ATPase. *Toxicol. Appl. Pharmacol.,* 109: 482-493

Hussain, T., Ali, M.M., Chandra, S.V., 1987. The combined effect of Pb2+ and Mn2+ on monoamine uptake and Na+, K+- ATPase in striatal synaptosomes. *J ApplToxicol.,* 7(4): 277-80.

James, C.K., Lai, Thomas, K.C., Leung, Louis Lim, Alex, W.K., Chan, Margaret J., Minski, 1991. Effects of chronic manganese treatment on rat brain regional sodium-potassium-activated and magnesium-activated adenosine triphosphatase activities during development. *Metabolic Brain Disease,* 6(3): 165-174

Kaur, J., Sharma, D., Singh, R., 1998. Regional effects of ageing on Na+, K(+)-ATPase activity in rat brain and correlation with multiple unit action potentials and lipid peroxidation. *Indian J BiochemBiophys.,* 35: 364- 371.

Kench, J. K., 1972. *Trans. Roy. Bio. Afr.,* 40: 209.

Lai, J.C.K., Guest, J.F., Leung, T.K.C., Lim, L., Davison, A.N., 1980. The effects of cadmium, manganese and aluminum on sodium-potassium-activated and magnesium-activated adenosine triphosphatase activity and choline uptake in rat brain synaptosomes. *Biochem. Pharmacol.,* 29: 141–146.

Lai, J.C.K., Guest, J.F., Lim, L., Davison, A.N., 1978. The effects of transition metal ions on rat brain synaptosomal amine-uptake systems. *Biochem. Soc. Trans.,* 6: 1010–1012.

Lai, J.C.K., Leung, T.K.C., Lim, L., 1984. Differences in the neurotoxic effects of manganese during development and aging: some observations on brain regional neurotransmitter and non-neurotransmitter metabolism in a developmental rat model of chronic manganese encephalopathy. *Neurotoxicology,* 5: 37–48.

Lai, J.C.K., Lim, L., Davison, A.N., 1981b. Differences in the inhibitory effects of Cd^{2+}, Mn^{2+} and Al^{3+} on the uptake of dopamine by synaptosomes from forebrain and from striatum of the rat. *Biochem. Pharmacol.,* 30: 3123–3125.

Lai, J.C.K., Lim, L., Davison, A.N., 1982c. Effects of Cd2+, Mn2+ and Al3+ on rat brain synaptosomal uptake of noradrenaline and serotonin. *J. Inorganic Biochem.,* 17: 215–225.

Moreira, E.G., Rosa, G.J., M., Barros, S. B.M., Vassilieff, V.S., Vassilieff, I., 2001. Antioxidant defense in rat brain regions after developmental lead exposure. *Toxicology,* 169: 145-151.

Reddy GR, Devi CB and Chetty CS., 2007. Developmental lead neurotoxicity: alterations in brain cholinergic system. *Neurotoxicology*., 28 (2): 402-407.

Sanui, H., Rubin, H., 1982. The role of magnesium in cell proliferation and transformation. In: Boynton AL, McKochan WL, Whitfield JP (eds). Ions, Cell Proliferation and Cancer. *Academic Press, New York.* 517–37.

Siegel, G.J., Fogt, S.K., Hurley, M.J., 1977. Lead action on sodium plus potassium activated adenosine triphosphatase from electroplax, rat brain and rat kidney. In: Eds, Shamoo, M. W. and A.E., Membrane Toxicity. *Plenum press, New York.* 465–493.

Siomara, C., Monteiro., *et al*. 2007. Supplementation with vitamins E plus C or soy isoflavones in ovariectomized rats: effect on the activities of Na^+, K^+-ATPase and cholinesterases. *Metabolic Brain Disease*, 22(2): 156–171

Tirri, R., Lagerspetz, K.Y.H., Kohnen, J., 1973. Temperature dependence of the ATPase activities in brain homogenates during the postnatal development of the rat. *Comp. Biochem. Physiol.,* 44B: 473.

Villalobos, V., Suarez, J., Estevez, J., Novo, E., Bonilla, E., 2001. Effect of chronic manganese treatment on adenosine tissue levels and adenosine A2 receptor binding in diverse regions of mouse brain. *Neurochem Res*., 26: 1157–61.

Walton, G.M., Gill, G.N., 1973. Adenosine 3', 5'-monophosphate and protein kinase dependent phosphorylation of ribosomal protein. *Biochemistry,* 3; 12 (14): 2604-2611.

Widzowski, D.V., Cory-Slechta, D.A., 1994. Homogenity of regional brain lead concentrations. *Neurotoxicology,* 15(2): 295- 307.

Wong, P.C.L., Lai, J.C.K., Lim, L., Davison, A.N., 1980. Selective inhibition of L-glutamate and gammar-aminobutyrale transport in nerve ending particles by aluminum, manganese and cadmium chloride. *J. Inorganic Biochem.,*14: 253–260.

Zaid, A., Gao, J., Squier, T.C., Michaelis, M.L., 1998. Age-related decrease in brain synaptic membrane Ca2+-ATPase in F344/BNF1 rats. *Neurobiol Aging.,* 19: 487 - 495.

****