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Region specific increase in the antioxidant enzymes and lipid peroxidation products in the brain of rats exposed to lead

CHRISTOPHER BENNET[1], RAJANNA BETTAIYA[1], SHARADA RAJANNA[1], LEVENIA BAKER[1], PRABHAKARA RAO YALLAPRAGADA[2], JON J. BRICE[1], SAMUEL L. WHITE[1], & KIRAN KUMAR BOKARA[2]

[1]*Department of Biological Sciences, Alcorn State University, Lorman, MS 39096, USA, and* [2]*Division of Animal Physiology & Toxicology, Department of Zoology, Andhra University, Visakhapatnam 530 003, India*

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Abstract

The objective of this study is to determine the effect of lead (pb) on antioxidant enzymes and lipid peroxidation products in different regions of rat brain. Wistar male rats were treated with lead acetate (500 ppm) through drinking water for a period of 8 weeks. Control animals were maintained on sodium acetate. Treated and control rats were sacrificed at intervals of 1st, 4th and 8th week and the whole brains were dissected on ice into four regions namely the cerebellum, the hippocampus, the frontal cortex and the brain stem. Antioxidant enzymes namely catalase and superoxide dismutase in all the four regions of brain were determined. In addition, lipid peroxidation products were also estimated. The results indicated a gradual increase in the activity of antioxidant enzymes in different regions of the brain and this response was time-dependent. However, the increase was more in the cerebellum and the hippocampus compared to other regions of the brain. The lipid peroxidation products also showed a similar trend suggesting increased effect of lead in these two regions of the brain. The data indicated a region-specific oxidative stress in the brain exposed to lead.

Keywords: *Lead toxicity, rat brain, antioxidant enzymes, lipid peroxidation products*

Introduction

Lead is a well known environmental pollutant and a neurotoxicant [1,2,3]. Lead contamination through air, dust, soil and water causes a broad range of physiological, biochemical and behavioral changes in central and peripheral nervous system [4,5]. Experimental studies have shown that lead induces a spontaneous release of neurotransmitters such as dopamine, acetylcholine, gamma aminobutyric acid (GABA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) [6–10]. A dose-dependent decrease in beta-adrenergic receptor density and adenylate cyclase activity was observed by Tsao et al. [11] in the brain of rats exposed to chronic levels of lead. Suszkiw [12] studied the effect of lead and found presynaptic disruption of transmitter release because of its interaction with voltage-gated calcium channels and calcium binding proteins that regulate the synaptic vesicle mobilization. Nihei and Guilarte [13] in a review on the possible molecular targets of lead have reported that lead alters neurotransmitters, hormones, transcription factors, receptors, enzymes and also the genes that code them. Struzynska et al. [14] and, Struzynska and Sulkowski [15] reported lead-induced

Correspondence: R. Bettaiya, Department of Biological Sciences, Alcorn State University, Lorman, MS 39096, USA.
Tel: 1 601 877 6681/1 601 638 5459. E-mail: brajanna@lorman.alcorn.edu; mbrajanna@yahoo.com

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disturbances in the synthesis and transport of neurotransmitters namely glutamate and GABA in rat brain. However, Gidlow [16] stressed the importance of lowering the occupational levels of lead exposure.

Lead mimics calcium and interferes at a number of stages in its cellular homeostasis including intracellular signaling [17–20]. Vazquez and Ortiz [21] reported that lead impairs long-term memory and cognition in adult rats by disturbing calcium related signaling mechanisms and protein kinase C in hippocampus. Lead exposure results in the hippocampal dysfunction [22], blockage of voltage-gated sodium channels in the hippocampus [23] and impair the long-term potentiation and memory [24]. Earlier research from our laboratory has demonstrated that lead causes perturbations in calcium homeostasis [25], *N*-methyl D-aspartate specific glutamate receptor binding [26] and ion regulation [27] in different regions of rat brain both *in vitro* and *in vivo* conditions. Lead was also shown to induce alterations in nitric oxide (NO) and nitric oxide synthase (NOS) of rat brain [28] and NO was well recognized as a second messenger to modulate signaling pathways of the brain [29]. A good correlation between NO and lead-induced cytotoxicity in PC-12 cells was reported by Sharifi et al. [30]. Recently, Chetty et al. [31] demonstrated lead-induced apoptosis in human neuroblastoma cells due to decrease in antioxidants and increase in caspase-3. Therefore, the disturbances caused by lead can be attributed to increase in reactive oxygen species in the brain; hence, the present investigation was undertaken. Moreover, the brain has a high rate of oxidative metabolism and high content of unsaturated lipids. The brain is also considered as a sensitive organ prone to oxidative damage because of its low levels of protective enzymes to eliminate free radicals [32]. It is also evident from the literature that both dose and exposure period to lead play an important role in causing oxidative stress [33–37]. Therefore, a second purpose of the present investigation was to determine that a dose of 500 ppm of lead acetate through drinking water in adult rats for a period of 8 weeks can cause oxidative stress. Since the same dose regimen (500 ppm) was followed earlier in all our investigations, a better correlation can be made with the data already collected on lead toxicity from our laboratory and also to understand the overall mechanism of lead toxicity.

Materials and methods

Chemicals

All biochemicals and lead acetate were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Treatment

Wistar male rats weighing approximately 100–120 g were obtained from Ghosh Enterprises, Calcutta (India). They were maintained in the animal facility for about 4 days before use. Rats were given a commercial feed (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. A group of 24 rats were exposed to 500 ppm of lead acetate in drinking water for a period of 8 weeks. Lead acetate was dissolved in distilled water and few drops (0.5 ml) of acetic acid were added to avoid precipitation. The control group (24 rats) received an equal amount of sodium acetate through drinking water. Sodium acetate was also dissolved in distilled water and the same amount of acetic acid was added. Both control and exposed groups were given free access to food. All treatments were stopped 6 h before sacrifice. The rats were sacrificed by cervical decapitation at intervals of 1, 4 and 8 weeks after treatment. At each interval, eight rats were used from each group of exposed and control. The whole brain was excised and immediately washed with ice-cold normal saline. Four regions of the brain namely the frontal cortex, the cerebellum, the hippocampus and the brain stem were isolated on ice. Due to small amount of tissue, the hippocampus from two brains was pooled for both exposed and control. The tissues were immediately used for the estimation of lipid peroxidation products, catalase and superoxide dismutase as described below:

Lipid peroxidation products

These products were estimated by the method of Hiroshi et al. [38]. Briefly, the homogenate (10%) of the different isolated tissues was prepared in 1.5% potassium chloride solution. One milliliter of the homogenate was added to 2.5 ml of 20% trichloroacetic acid (TCA). The mixture was centrifuged at 3,500 rpm for 10 min at 4°C. The pellet was then dissolved in 2.5 ml of 0.05 M sulphuric acid and 3 ml of 2 M thiobarbituric acid was added to it. The test tubes were incubated in boiling water bath at 100°C for 30 min. The contents were cooled and the color was extracted into 4 ml of *n*-butanol. The color was read at 530 nm using a spectrophotometer against the blank. The results were expressed as micromoles of MDA formed/gm weight of tissue.

Catalase

The enzyme activity was determined by the method of Aebi and Packer [39]. Different regions of the brain were homogenized separately in 0.05 M phosphate buffer (pH 7) containing 0.1 mM EDTA. The homogenate (10%) was centrifuged at 4000 rpm for 15 min at 4°C. The supernatant was decanted and centrifuged at 16,000 rpm for 60 min at 4°C. The

supernatant was used for the enzyme assay. The reaction mixture consisted of 100 µl of supernatant and 10 µl of alcoholic ethanol. This was vortexed well and then kept on ice water bath for 10 min. The tubes were brought to room temperature and 10 µl of Triton X-100 RS was added and vortexed well till the whole tissue extract was completely dissolved. Then, a freshly prepared hydrogen peroxide (H_2O_2) solution (0.66 M in phosphate buffer) of 100 µl was added to the above reaction mixture and the decrease in absorbance was read in a spectrophotometer at 240 nm against a blank for 60 s. All reactions were carried out in darkness. The data were expressed as micromoles of H_2O_2 metabolized/mg protein/min.

Superoxide dismutase

The enzyme assay was performed by using the method described by Beauchamp and Fridovich [40]. A 10% homogenate was prepared using a homogenizing buffer (100 mM phosphate buffer, pH 7.5) and then centrifuged at 4,000 rpm for 10 min at 4°C. The supernatant was decanted and then centrifuged at 16,000 rpm for 60 min at 4°C and the supernatant was used for the assay. The reaction mixture contained 1.5 ml of 100 mM phosphate buffer (pH 7.5), 0.3 ml of 130 mM methionine, 0.3 ml of 750 mM nitroblue tetrazonium (NBT), 0.3 ml of 10 mM EDTA and 0.1 ml of enzyme source (supernatant). The reaction was started by addition of 0.1 ml of freshly prepared riboflavin (60 mM). After the addition of riboflavin, the tubes were placed under light for 30 min. A similar set of controls was maintained under dark condition. The optical density was read at 560 nm against the controls kept in dark condition. The results were reported as units/mg protein. One unit of enzyme activity was defined as the amount of enzyme that decreases the initial rate to 50% of its maximal value for the particular tissue being assayed.

Statistics

All assays were done in triplicate. The mean ($n = 8$) and standard deviations were calculated using standard statistical procedures. Comparison between control and exposed was made by following Student's "*t*" test [41] and significant differences were calculated at $p < 0.05$.

Results

The results on lipid peroxidation products in four different tissues of the rat brain namely the cerebellum, the hippocampus, the frontal cortex and the brain stem were presented in Figure 1. There was an increasing pattern of lipid peroxidation products in all four tissues of the brain with increasing period of exposure but there were variations in relation to exposure period indicating tissue specificity (Figure 1). There was a gradual and significant ($p < 0.05$) increase of lipid peroxidation products in the cerebellum from 1st to 8th week (Figure 1) in exposed rats when compared to their respective controls. The highest amount of increase was observed in the hippocampus at 1st week and a moderate increase at 8th week of exposure in comparison to their respective controls. However, the increase was significant ($p < 0.05$) at 4th week of exposure in this tissue. The frontal cortex also had a gradual and moderate increase in the peroxidation products from 1st to 8th week (Figure 1) with significant ($p < 0.05$) values at the intervals of 4th and 8th week of exposure when compared to their respective controls. A gradual and less amount of increase was noticed in the brain stem with increasing exposure period and the increase was significant ($p < 0.05$) only at 8th week of exposure in comparison to their control. The rate of increase in lipid peroxidation products was in the order of the hippocampus > the cerebellum > the frontal cortex > the brain stem.

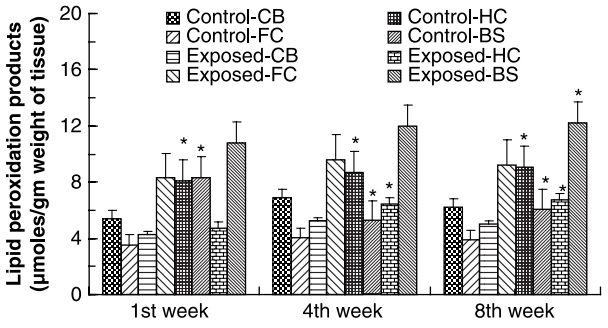

Figure 1. Lipid peroxidation products in different regions of brain in control and exposed rats. Each value represents the mean ± standard deviation ($n = 8$). The expermental details are given in Materials and methods. *Significantly different from its respective control at $p < 0.05$.

Catalase activity was presented in Figure 2 for different tissues of the brain. The cerebellum showed an initial high and significant ($p < 0.05$) increase in the catalase activity in the 1st and 8th week of exposure. However, in comparison to control, the increase at 4th week was not significant ($p > 0.05$) (Figure 2). The levels of catalase activity were found to be gradually increasing with the increasing period of exposure in the hippocampus and this increase was also significant ($p < 0.05$) at all intervals in comparison to their controls. The frontal cortex showed a gradual and significant ($p < 0.05$) increase in catalase activity with the increasing period of exposure compared to their respective controls. A moderate and significant ($p < 0.05$) increase in the catalase activity was noticed in the brain stem at different intervals of exposure (Figure 2). The rate of increase in catalase activity of different tissues of the brain is in

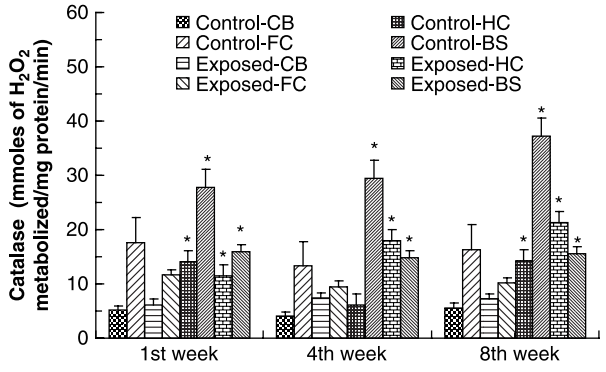

Figure 2. Catalase activity in different regions of brain in control and exposed rats. Each value represents the mean ± standard deviation ($n = 8$). The experimental details are given in Materials and methods. *Significantly different from its respective control at $p < 0.05$.

the following order: the frontal cortex > the hippocampus > the cerebellum > the brain stem.

Figure 3 represents the data on superoxide dismutase for four different tissues of brain. Cerebellum showed a high amount of increase in superoxide dismutase activity both at 1st and 8th week of exposure and the increase was significant ($p < 0.05$) with respect to their controls. However, the enzyme activity was moderately higher and significant ($p < 0.05$) at 4th week of exposure in this tissue (Figure 3). A gradual and significant ($p < 0.05$) increase in the enzyme activity was noticed in the hippocampus with increasing period of exposure. The frontal cortex had a high amount of increase in enzyme activity at 4th and 8th week of exposure but a moderate increase was observed at 1st week. Nevertheless, the activity was found to be significantly ($p < 0.05$) high in this tissue at all intervals. There was a less amount of increase in superoxide dismutase activity in the brain stem but found significant ($p < 0.05$) in comparison with their respective

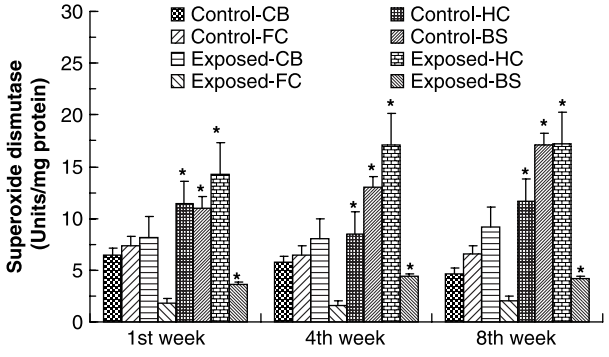

Figure 3. Superoxide dismutase activity in different regions of brain in control and exposed rats. Each value represents the mean ± standard deviation ($n = 8$). The experimental details are given in Materials and methods. *Significantly different from its respective control at $p < 0.05$.

controls. The following is the order in rate of increase in enzyme activity for different tissue of the brain: the frontal cortex > the hippocampus > the cerebellum > the brain stem.

Discussion

The results indicate a differential response to lead in all the four regions of rat brain for lipid peroxidation products and antioxidant enzymes, catalase and superoxide dismutase. Earlier investigations [42] have reported lipid peroxidation and oxidative stress in the whole brain with chronic treatment of high amounts of lead (1,000 ppm). According to Gong and Evans [43], a dosage of 2,000 ppm of lead through drinking water for 21 days to rats resulted in more neuronal damage than with 150 ppm. But the present investigation has proved that chronic treatment of lead using 500 ppm of lead causes increase in lipid peroxidation products and oxidative stress. However, the present data on lipid peroxidation products may not support the time-dependent increase in lead-induced damage but there appears to be region-specific response of lead and this might be due to either differential accumulation of lead in various regions of brain as has been observed earlier (Abstract, National Minority Research Symposium, Cancun, Mexico, 2001) or because of differential susceptibility of cells to lead in each region of brain [44–46] or the differences in fatty acid content [34]. It was evident that there was more increase in the peroxidation products in the hippocampus compared to other regions of the brain suggesting that hippocampus is more susceptible to lead-induced neurotoxicity compared to other regions of the brain. The peroxidation products in cerebellum were higher than the frontal cortex and brain stem indicating that this is also vulnerable to lead toxicity. Similar lead induced differential effects of oxidative stress were reported by Sandhir et al. [33] and, Jindal and Gill [47]. Lead induced oxidative stress results from inhibition of 5-aminolevulinic acid (ALA) dehydratase and its direct interaction with biological membranes inducing lipid peroxidation. Similar lead-induced disruption by free radicals was reported by Jiun and Hsien, [48] and West et al. [49]. Our results also corroborate well with that of Bachara et al. [50] Oteiza et al. [51] and Adanaylo and Oteiza [42] all of whom have demonstrated that lead increases the rate of lipid peroxidation.

Lead-induced effects on antioxidant defense might cause impairment in pro-oxidant/antioxidant balance of cell leading to oxidative injury [52,53]. In the present study, a significant increase was found in antioxidant enzymes in all the four regions of rat brain with differential response indicating tissue specific toxicity against lead. Similar involvement of antioxidant enzymes during oxidative stress was reported in

rat brain exposed to paraquat [54] light exposure [55] and Enalapril treatment [56]. Monteiro et al. [57] and Gurer et al. [58] suggested an auto oxidation of excessively accumulated aminolevulinic acid dehydratase due to lead inhibition which may result in the formation of superoxide and H_2O_2. According to Halliwell [59], participation of lead with iron in Fenton reaction leads to the production of more reactive hydroxyl radicals from superoxide and H_2O_2 resulting in the increased lipid peroxidation. Other metals like aluminum [60], cadmium [61], mercury [62] and thallium [63] can also induce oxidative stress producing lipid peroxidation products in different tissues. Similar region-specific accumulation of metals was reported for lead [64,65], cadmium [66], manganese [67], thallium [63], copper, zinc and iron [65].

Catalase is one of the major antioxidant enzymes having heme as the prosthetic group. In this study, results from the hippocampus showed an increase in the catalase activity at all the three intervals of the exposure period. A comparison of results in all the four regions of the brain showed that the frontal cortex had high increase in catalase activity in the initial and final stages, that is in the 1st and 8th week, whereas there was little increase at 4th week which was not statistically significant at 4th week suggesting that free form of lead might have interfered with the tissues in the initial and final stages. While during the 4th week, some of the cells in the frontal cortex which might be rich in SH groups, interacted with the free lead showing less oxidative stress. Superoxide dismutase plays an important role in protecting the cells against oxidative stress by catalyzing its dismutation reaction. The data on superoxide dismutase had differential levels of increase in the levels of enzyme activity in all the four regions of rat brain at all the intervals, however the highest increase was observed in the frontal cortex indicating the sensitivity of the region towards lead-induced toxicity.

Catalase and superoxide dismutase are metalloproteins and accomplish their antioxidant functions by enzymatically detoxifying peroxides, H_2O_2 and oxygen, respectively, since these antioxidant enzymes depend on various essential trace elements for proper molecular structure and enzymatic activity but are the potential targets for lead toxicity as has been reported by Gelman et al. [68]. Selenium is associated with antioxidant enzymes including superoxide dismutase and this selenium gives protection against lead toxicity [69]. The protective role of alpha tocopherol, ascorbic acid, *N*-acetylcysteine, L-methionine and curcumin clearly demonstrates the effect of lead-induced oxidative stress [35,52,70]. According to Hsu and Guo [52], supplementation of antioxidants prevents lead-induced oxidative stress and this indirectly supports our results that lead enhances antioxidant enzymes.

These results on enhanced levels of lipid peroxidation products and antioxidant enzymes also support our earlier findings of increased intracellular calcium levels and nitrous oxide [25,28] due to lead-induced oxidative stress with the same dose regimen. Similar results have been reported in rat brain neurons by Oyama et al. [71]. Though lead-induced pathological changes were investigated in the present study, several investigators [31,72] have already reported cell death and apoptosis lead administration. Other metals like cadmium [73], aluminum [74] and toxins [75] can also cause neuronal cell death. In our laboratory, further investigations are in progress to understand the influence of antioxidant enzymes namely glutathione peroxidase, glutathione reductase and glutathione (oxidized and reduced) in different regions of the brain with the same treatment protocol.

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