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ORIGINAL ARTICLE

Neurochemical and Neurobehavioral Effects of Low Lead Exposure on the Developing Brain

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Abstract Lead is found in small but appreciable quantities in air, soil drinking water and food. Exposure to such amounts of lead does not cause acute lead toxicity, but produces subtle effects, particularly in children. The CDC advocates "safe" or "acceptable" levels of blood lead up to 10 μg/dl, while OSHA declares blood lead levels up to 40 μg/dl as "safe" or "acceptable" in the occupationally exposed. The objective of the study was to see if blood levels considered "safe" can cause changes in the biogenic neurotransmitters in the developing brain which may cause neurobehavioral defects like hyperactivity and other cognitive disorders. Albino Wistar rats were divided into the control and lead-treated groups. The control group was given unleaded water, while the lead-treated group was fed with 50 ppm lead acetate in drinking water. On day 45 the animals were subjected to a passive avoidance test, their blood analysed for ZPP and lead. They were then sacrificed and the neurotransmitters—Norepinephrine (NE) and its metabolite—methoxyhydroxyphenylglycol (MHPG) estimated in the brain areas associated with learning and memory—the frontal cortex, hippocampus and the striatum by HPLC-ECD. Our results showed significant increases in blood lead, NE and MHPG, while ZPP increase was insignificant. The rats showed neurobehavioral abnormalities as assessed by the passive avoidance test. We concluded that low blood levels of lead cannot be considered "safe" or "acceptable" as it causes neurotransmitter alterations. Increased NE turnover is implicated in hyperactivity disorders such as ADHD and Tourette syndrome.

Keywords Lead · Norepinephrine · Neurobehavioral · Frontal cortex · Hippocampus · Striatum

Abbreviations

CDC	Centre for Disease Control
OSHA	Occupational Safety and Health Administration
NE	Norepinephrine
MHPG	3-Methoxy-4-hydroxyl phenyl glycol
ZPP	Zinc protoporphyrin

Introduction

Lead is the 5th most abundant metal in the world after iron, copper, zinc and aluminum [1]. Lead has many uses—for production of ammunition, bearing metals, brass and bronze, cable covering, extruded products, sheet lead, and solder, used in ceramics, type metal, ballast or weights, tubes or containers, oxides, and gasoline additives. Human exposure to lead occurs primarily through diet, air, drinking water, dust, and paint chips. Lead-based paint remains the most common high-dose source of lead exposure for preschool children. Children may be exposed to high lead levels when workers take home lead on their clothing or when they bring scrap or waste material home from work [2]. Absorption may vary depending on dietary factors and the chemical form of the lead. Lead is absorbed into the body following ingestion and inhalation exposure. Adult humans absorb 10–15% of ingested lead; however, children absorb up to 50% of ingested lead. Absorption is also increased in children suffering from iron or calcium deficiencies [3].

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The toxic effects of lead in humans have been well documented. Nonspecific signs and symptoms of lead intoxication include loss of appetite, metallic taste, constipation, pallor, malaise, weakness, insomnia, headache, irritability, pain in muscles and joints, fine tremors, colic, and Burton's lines (purple-blue discoloration of the gums). Specific effects occur in target organs and systems: nervous system (central and peripheral), hematopoietic system, cardiovascular system, kidneys, reproductive system, and fetus.

The most critical effects of lead toxicity occur among children exposed during fetal development, postnatal development, or both [1]. Children are much more sensitive to lead intoxication than adults. In children, encephalopathic symptoms and death occur at blood lead concentrations of 80–100 μg/dl. Overt subencephalopathic symptoms (peripheral nerve damage) occur at blood levels of 40–60 μg/dl [4]. Non-overt neurotoxic effects [evidenced by slowed nerve conduction velocities and cognitive (IQ), electrophysiological, and neuropsychological defects] were detected in children at levels less than or equal to 40 μg/dl, with no clear threshold being evident [4].

Lead is a highly reactive divalent cation. It exerts its toxic effects because of its high affinity for proteins, especially at sulfhydryl groups. Lead also inhibits Na–K ATPase at levels as low as 20 μg/dl. Lead mimics the action of Ca^{++} (at much lower concentrations than calcium) by direct stimulation of protein kinase 'C' which phosphorylates proteins, and by stimulation of cAMP dependent protein kinases.

Aims and Objectives

The objective of the present study was to evaluate the effect of pre and postnatal low level lead exposure on the levels of blood lead and on the neurotransmitter—Norepinephrine (NE) and its metabolite—methoxyhydroxyphenylglycol (MHPG) in the developing brain of mice. The study also aimed at the assessment of the effect of these neurotransmitter alterations on learning and memory.

Materials and Methods

Experiments were carried out on Wistar Albino mice. The animals were divided into 2 groups—control and lead treated. The control group was maintained on unleaded and deionised water while in utero (fed to the pregnant dam) and up to 45 days, while the lead treated mice were on 50 ppm lead acetate in drinking water, while in utero and up to 45 days. After weaning, both the groups were on commercially available rat feed ad libitum. On day 45 the experimental animals were weighed, subjected to a passive avoidance test to assess their neurobehavioral faculty, their blood analysed for ZPP and lead. They were then sacrificed and the neurotransmitter—NE and its metabolite—MHPG were estimated in three brain areas-the frontal cortex, hippocampus and the striatum zinc protoporphyrin was measured using a Haematofluorometer and expressed in μg/dl [5].

Blood lead was estimated using the 3010B ESA lead Analyser which is based on the principle of Anode Stripping Voltammetry. It is expressed as concentration of lead in blood in μg/dl [6].

Neurobehavioral faculty was assessed using the Step through passive avoidance test [7].

NE and its metabolite, MHPG were estimated by high performance liquid chromatography with electrochemical detection [8].

Results and Discussion

Table 1 shows the blood lead levels and the zinc protoporphyrin levels in control and lead treated mice. From the table we see significant increase in the blood lead levels of lead treated mice ($P < 0.005$). For the control the mean blood lead levels was 4.06 ± 0.077 μg/dl and for the lead treated mice, it was 10.65 ± 3.09 μg/dl. The zinc protoporphyrin levels on the other hand show insignificant increase ($P > 0.005$). The ZPP levels in controls was 16.80 ± 6.55 μg/dl and in lead treated mice it was 27 ± 8.27 μg/dl.

Table 2 shows the levels of NE in the control and lead treated mice on day 45. In the frontal cortex, NE shows an increase but not a significant one ($P > 0.005$); while in the hippocampus and striatum, there is a significant increase ($P < 0.005$). The controls had mean value of 7.35 ± 2.09 ng/g in the frontal cortex, 6.47 ± 2.00 ng/g in the hippocampus and 4.65 ± 2.21 ng/g in the striatum while the lead treated mice had mean values of 9.25 ± 2.91 ng/g body weight in the frontal cortex, 15.55 ± 2.65 ng/g in the hippocampus and 21.92 ± 5.85 ng/g weight in the striatum.

Table 3 shows the levels of MHPG—which is the metabolite of NE, in the 3 brain regions. From the table we can see significant increase in the levels of MHPG in all three areas of

Table 1 Blood lead and ZPP levels of control and lead exposed rat pups on days 45

	Control	Lead treated	*P* value
Blood lead in μg/dl (mean ± SEM)	4.06 ± 0.77	10.65 ± 3.09	0.001**
ZPP in μg/dl (mean ± SEM)	16.8 ± 6.55	27 ± 8.27	0.01

* Statistical significance at 5%

** Statistical significance at 1%

Table 2 Effect of lead on NE in three area of brain

	Control (mean ± SD) ng/g	Subjects (mean ± SD) ng/g	Student *t*	*P* value
Frontal cortex	7.35 ± 2.09	9.25 ± 2.91	1.126	0.289
Hippocampus	6.47 ± 2.00	15.55 ± 2.65	3.641	0.000**
Striatum	4.65 ± 2.21	21.92 ± 5.85	6.197	0.000**

* Statistical significance at 5%

** Statistical significance at 1%

the brain ($P < 0.005$). In the controls—MHPG levels were 63.02 ± 10.25 ng/g weight in the frontal cortex, 58.06 ± 18.70 ng/g weight in the hippocampus and 158.40 ± 25.88 ng/g weight, in the striatum. In the lead treated mice the levels were 123.86 ± 21.72 ng/g weight in the frontal cortex 127 ± 34.81 ng/g weight in the hippocampus and 660.14 ± 132.28 ng/g weight in the striatum.

The data presented in Table 4 shows that the median baseline (before the electric shock) step through latency score for the controls on day 45 is 2 s while that for the lead treated animals is 14 s. This indicates a deficit in the basic learning and memory functions much before the exposure to the aversive event. The lead treated rats took a much longer time to recall the way to the dark chamber and enter into the dark chamber when they were placed in the illuminated chamber as against the unleaded control animals which entered the dark chamber almost immediately after being placed in the illuminated chamber.

When we see the final latency (after the electric shock) score, we find that the lead treated animals have a much shorter latency period the median being 34 s in the lead treated subjects, as compared to the controls which had a median of >180 s (cut-off time). This indicates that the lead treated animals failed to remember the electric shock that they had received in the dark chamber and therefore entered the dark chamber earlier than the control animals.

Discussion

Lead is such a widely used metal, that lead toxicity is one of the most commonly seen occupational health hazard. The levels of lead considered to be safe have been continually lowered in the past decades, and it has been proved that a threshold for damage due to lead may not exist. The adverse effects on human beings of prenatal exposure to low levels of lead are unknown, since manifestation of toxic effects may not appear until years after the initial insult. Exposure to lead has been suggested as a cause of hyperactivity in children. [9]. The objective of the present study was to evaluate the effect of pre and postnatal low level lead exposure on the neurotransmitter—NE and its metabolite—MHPG developing brain of mice. We see that blood lead levels increase significantly with low lead exposure (Table 1), while the increase in ZPP is insignificant, suggesting that ZPP and blood lead levels do not correlate well. On day-45 there is an increase in the levels of NE, in the frontal cortex, and it is evident that with a 1.5 fold increase in blood lead levels, the levels of NE in the frontal cortex has increased, but not significantly (Table 2). Similarly in the hippocampus the levels of NE have increased. In the striatum too, we see NE levels increasing significantly.

Table 4 Baseline and final latency score in control and lead treated mice

Step through latency score	Control ($n = 10$)	Lead treated ($n = 10$)
Baseline latency score (median) (in seconds)	2.0	14.0
Final latency score (median) (in seconds)	>180	34.0

Metabolism of Norepinephrine

NE is synthesized from tyrosine by hydroxylation by tyrosine hydroxylase, which uses tetrahydrobiopterin as the cofactor. The product, dihydroxyphenylalanine (DOPA), is converted to dopamine through the action of Dopa decarboxylase [10]. Dopamine-β-hydroxylase is responsible for final conversion of dopamine to NE, which is then stored. Two important enzymatic events for the inactivation of catecholamines involve catechol-*O*-methylation and oxidative deamination by the enzymes—COMT and MAO respectively. Most of the catecholamines undergo deamination by MAO to 3-methoxy-4-hydroxymandelic aldehyde which is rapidly oxidized to vanillylmandelic acid (VMA) or reduced to MHPG, both of which are subsequently excreted. MHPG is the major metabolic end product of NE [10].

Table 3 Effect of lead on MHPG in three area of brain

	Control (mean ± SD) ng/g	Subjects (mean ± SD) ng/g	Student *t*	*P* value
Frontal cortex	63.02 ± 10.25	123.86 ± 21.72	5.664	0.000**
Hippocampus	8.06 ± 18.70	127.00 ± 34.81	3.955	0.003**
Striatum	158.40 ± 25.88	660.14 ± 132.28	8.278	0.000**

* Statistical significance at 5%

** Statistical significance at 1%

Release of the stored neurotransmitter is brought about by the Ca^{++} dependent phosphorylation of the cytoskeleton protein like synapsin I [11]. Lead mimics the action of Ca^{++}. (at much lower concentrations than calcium) [12]. Thus, low levels of lead release NE from the secretory vesicles, by bringing about phosphorylation of proteins of the cytoskeleton, similar to what Ca^{++} does. This results in increase NE levels and therefore an increase in the NE turnover. Lead acts, by direct stimulation of protein kinase 'C' and also by the stimulation of cAMP dependent protein kinases.

Lead also behaves as a chemical stress. Prolonged stress causes prolonged presynaptic activity and stimulates tyrosine hydroxylase resulting in increased NE synthesis and release. This is probably why we see that NE levels increase in all the three regions of the brain. The hippocampus is a target of chronic stress and is affected by repeated stress as observed by Sapolsky [13] and McEwen [14]. Moreover, it has been observed that with prolonged stress, the feedback inhibition on tyrosine hydroxylase by NE is lost. Both these mechanism may be responsible for high levels of NE in all the 3 brain regions. A look at the levels of MHPG, which is a metabolite of NE, shows very large increases indicating an increase in the breakdown of NE to MHPG in all the three brain regions. Lead is believed to stimulate and increase the activity of MAO, as shown in studies carried out by Flora and Seth (2000), who demonstrated an increase in the activity of the monoamine oxidase system on chronic exposure to lead [15]. Increased activity of MAO enzyme results in increased metabolism or breakdown of NE to MHPG. Also prolonged stress, as in prolonged exposure to lead, causes rapid turnover of NE [16].

Bressler and Goldstein have described in their work, the mechanism of lead toxicity [17]. They suggested that at the neuronal level, lead alters the release of neurotransmitters from the presynaptic nerve endings. Spontaneous release of neurotransmitters is enhanced [17]. Golter and Michaelson have also demonstrated a small but significant increase (13%) in brain NE [18].

Shailesh Kumar and Desiraju in their studies on the regional alterations of brain biogenic amines and GABA/glutamate levels in rats following chronic lead exposure (60 days) during neonatal development have shown elevation of NE in the hippocampus, cerebellum, hypothalamus, brain steam and nucleus accumbens striatum [19].

Results of the passive avoidance test shows that neurobehavioral alterations occur even with low lead exposure. This is clearly seen as delay in the basal latency period (Table 4) which indicates slow learning process. The shortening of the final latency period shows that the leaded rats failed to remember the aversive event (Table 4). Studies such as those of Rosetti et al. have linked elevated catecholamine levels with learning and memory defects [20]. It has been well demonstrated that increase in NE levels produce amnesia [21, 22]. Low lead levels have been demonstrated to effect different regions of the brain and different neurotransmitters [23]. Shailesh Kumar and Desiraju (1990) have put forth an interesting conclusion derived from their study involving regional alterations in brain biogenic amines after chronic exposure to lead [19]. They attribute the differences observed in the neurotoxic effects of lead in the different regions for the transmitters, to the vulnerability of the axon terminals of any given type which are dependant on some regional factors, although the projections of the different regions originate form an apparently similar category of neurons in the brain stem [19].

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