Effect of Lead Exposure on Dopaminergic Transmission in the Rat Brain

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ABSTRACT

Lead is a neurotoxicant with known behavioral and neurochemical effects. In this study we attempted to relate the behavioral effects of lead to neurotransmission. Oral administration of 1000 ppm of lead acetate to young rats for 30 days caused a reduction in locomotor activity and stereotypic exploratory behavior during a 20 minute testing period. This locomotor hypoactivity induced by lead was accompanied by a reduction in stereotypic behavior (sniffing, lickings, biting and grooming). These outcomes suggested that lead may interfere with catecholaminergic and particularly dopaminergic neurotransmission. Therefore we examined the effect of the lead acetate on the uptake of dopamine in striatal synaptosomal preparations. The collected data showed a clear inhibition of the uptake of ^{3}H-DA with an IC50 of 3.5×10^{-5} M. This inhibition of the uptake of dopamine suggests that the behavioral effects of lead may be involved in dopaminergic neurotransmission. **(Int. J. Ch. Neuropsychiatry, 2004, 1(1): 97-105)**

INTRODUCTION

Lead is a polluting agent of the ecosystem and is introduced into the food chain by various mechanisms[1,2]. Lead poisoning is, and for centuries has been, one of the most significant preventable causes of neurological morbidity from an environmental toxin. As a heavy metal, lead is ubiquitous in our environment, yet it has no physiologic role in biological systems. Its effects are pervasive and often subtle, with consequences ranging from cognitive impairment in children to peripheral neuropathy in adults. While occupational exposure among workers at smelters or battery recycling plants remains an occasional problem, the greatest public health problem at the present time is exposure of young children to decaying fragments of leaded paint. The mechanism by which lead disrupts normal physiological processes is based on the similarity of ionized lead (Pb^{++}) to calcium (Ca^{++}). Both are divalent cations; however, Pb^{++} can disrupt the physiological effects of Ca^{++} at concentrations several orders of magnitude lower than the concentration of Ca^{++}. In the developing brain, Pb^{++} causes an inappropriate release of neurotransmitters at rest and competes with Ca^{++} to interfere with evoked neurotransmitter release. This increase in basal release and decrease in evoked release may interfere with selective pruning of synaptic connections in the brain

during the first few years of brain development.

Lead also interferes with excitatory neurotransmission by glutamate, which is the transmitter at more than half the synapses in the brain and is critical for learning. The glutamate receptor thought to be associated with neuronal development and plasticity is the N-methyl-D-aspartate (NMDA) receptor, which is blocked selectively by lead. This disrupts long-term potentiation, which compromises the permanent retention of newly learned information.

Lead causes activation of protein kinase C (PKC) and binds to PKC more avidly than Ca^{++}, its physiologic activator. Alteration of PKC function compromises transmitter and second-messenger systems within the cell, leading to further changes in gene expression and protein synthesis.

At higher blood levels, Pb^{++} disrupts the function of endothelial cells in the blood-brain barrier. This may lead to hemorrhagic encephalopathy, characterized by seizures and coma.

Lead has an effect on heme biosynthesis, causing anemia at high blood levels; however, at low levels, Pb^{++} causes microcytosis (ie, decreased mean corpuscular volume [MCV] and mean corpuscular hemoglobin [MCH]) and a compensatory increase in number of red blood cells. Lead irreversibly binds to the sulfhydryl group of proteins, causing impaired function without any discernible threshold. The enzymes delta-aminolevulinic acid dehydratase, which catalyzes the formation of the porphobilinogen ring, and ferrochelatase, which inserts iron into the protoporphyrin ring, both are compromised by lead.

We arranged this present work to evaluate if lead exposure would interfere with the uptake of dopamine in striatal synaptosome preparations and/or would cause alterations in dopamine-dependent.

MATERIALS AND METHODS

Animals Exposure

Thirty young male Wistar rats, 3 weeks old and weighing approximately 45-50g at the beginning of the experiment were used. The rats were housed in an animal facility in plastic cages at an ambient temperature of 22 ± 1°C and photoperiod (12 h light/dark cycle) and relative humidity was usually between 50 and 60%. Access to water and food WAS libitum. The amount of food consumed was monitored, food was restricted at certain periods of the day during behavioral testing. Lead acetate (1000ppm dissolved in distilled water) was given to rats in drinking water for 30 days. Rats were weighed on the 7^{th}, 14^{th}, 21^{st} and 30^{th} days of exposure.

Behaviour testing

Each rat was placed in a cylindrical cage. The stereotypic behavior of each rat was observed and noted every 5 minutes during 20 minutes (namely: sniffing, licking, bite and grooming). Stereotypic behaviors were measured by allotting to each observation a score of 1 for each animal continuously sniffing, biting, licking or cleaning. A score of 0 was assigned to the absence of these stereotypic behaviors. The sum of the scores allotted to each observation was then calculated for each behavior, and the average relative to each rat was calculated. As for the locomotor activity, it was characterized by horizontal activity of the animals in the cage during a 20 minute experimental period.

Tritiated dopamine uptake

The animals treated with lead acetate for 30 days and controls were decapitated at an age of 8 weeks, brains were removed, and subsequently, the homogenates were prepared from striatal tissue using a Potter Elvehyem homogenizer. The homogenization was carried out in 10 volumes of frozen sucrose solution (0.32 M), at 800 ± 50 rpms, for 20 seconds at 4°C. The homogenate was centrifuged at1000g for 10 minutes at 4°C. The supernatant (synaptosomal fraction) was kept on ice in the presence of 2 µM of pargyline (to inhibit monoamine oxidases) and was incubated at 37°C for 5 minutes with 20 nM ^{3}H-DA. The reaction was stopped by dilution with 3 ml of the incubation buffer and filtered on glass fiber filters (hattman GF/B 25 mms diameter). The filters were rinsed 2 times with 3 ml of buffer.

In in vitro experiments, lead acetate was added to the incubation medium containing synaptosomes obtained from control rats. in the presence of various lead acetate concentrations (10^{-3}M with 10^{-9}M). Specific uptake was defined as the difference between the sum of uptake (at 37°C) and specific uptake (at 0°C). Results were expressed in fentomoles of collected dopamine (mg/protein) and the inhibitory concentration (IC50) for lead was calculated. Protein concentrations were determined by the method of (3)

RESULTS

The administration of lead acetate causes a significant reduction in locomotor activity during a 20 minute trial period as compared to the controls (Fig. 1). This locomotor hypoactivity effect is accompanied by a very significant decrease in stereotypic behaviors of the animals (Figs. 2, 3, 4). These results show that lead induces a locomotor hypoactivity throughout the trial period and disturbs stereotypic and exploratory behavior of the animals (sniffing, lickings and grooming). This study related the effect of the lead acetate to the dopaminergic neurotransmission. We observed that the addition of various lead acetate concentrations to synaptosome preparations of strialale origin, produced an inhibition of the uptake of triated dopamine (3H-DA) (Fig. 5), with an IC50 about 3, 5.10^{-5}M.

In the same way, we observed in other series of experiments, that the lead acetate administration as increasing concentrations (Fig. 6) throughout 90 days, a clear inhibition of the uptake of 3H-DA with an dose-effect proportions.

To look further into the interaction of lead on the dopaminergic ways, we studied the effect of this metal on the digestive behavior of the rat. Knowing that many cerebral structures are implied in the regulation of the processes digestive and that the actions of growth regulators observed at the time of a given treatment take support to a large extent on the catecholaminergic transmissions[4].

The results presented in table (1), show a modification in the gain of body weight between the Pb- treated rats and the controls rats. The gain of weight decreased gradually from one week to another during the whole period of the lead acetate administration.

Table 1. Effect on body weight development in rats exposed to 1000ppm lead in drinking water, the values are expressed in averages ± SEM of 7 determinations by tests.

	B.W initial(g)	7th day	14th day	21th day	30th day
Rats controls(0ppm)	101.30± 2.99	120.86± 4.70	143.80± 4.33	178.00± 4.56	200.40± 3.90
Rats exposed(1000ppm)	101.30± 2.99	123.00± 1.00	141.50± 1.64	153.50± 2.85	161.70± 4.18

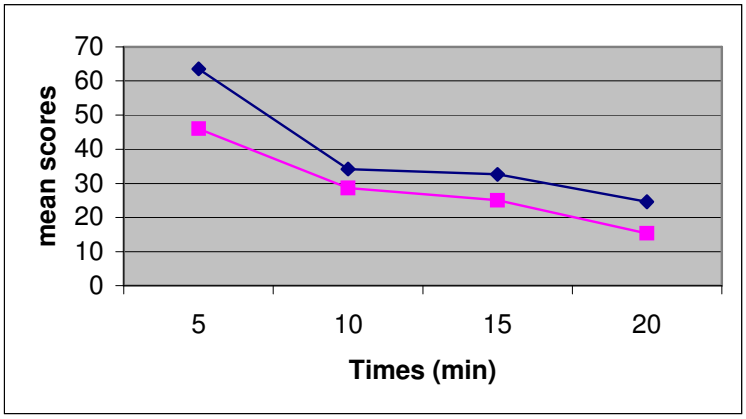

Fig. (1): The effect of lead acetate on locomotor activity in controls ◊ and lead-exposed rats □. Behavioral tests were conducted as specified in the methods section. Values represent the means of 5 experiments; SD less than 5%.

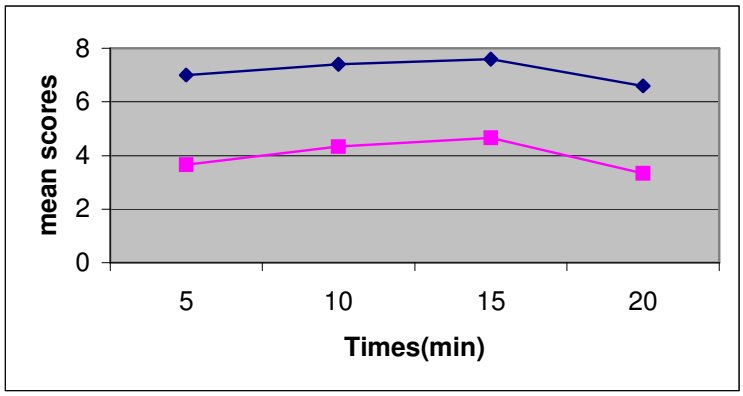

Fig. (2): The effect of lead acetate on stereotypic activity (sniffing) in controls ◊ and lead-exposed rats □. Behavioral tests were conducted as specified in the methods section. Values represent the means of 5 experiments; SD less than 5%.

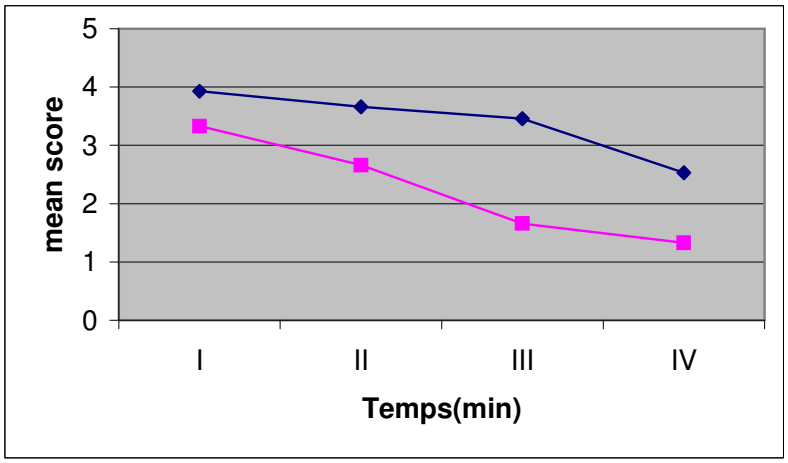

Fig. (3): The effect of lead acetate on stereotypic activity (licking) in controls ◊ and lead-exposed rats □. Behavioral tests were conducted as specified in the methods section. Values represent the means of 5 experiments; SD less than 5%.

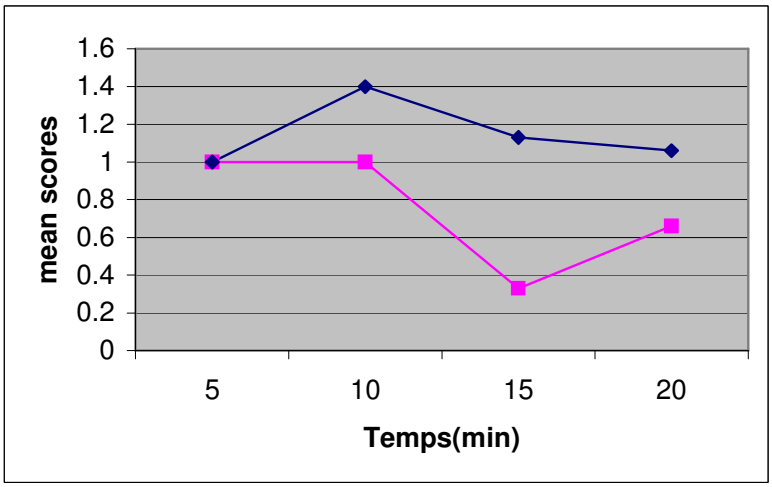

Fig. (4): The effect of lead acetate on stereotypic activity (cleaning) in controls ◊ and lead-exposed rats □. Behavioral tests were conducted as specified in the methods section. Values represent the means of 5 experiments; SD less than 5%.

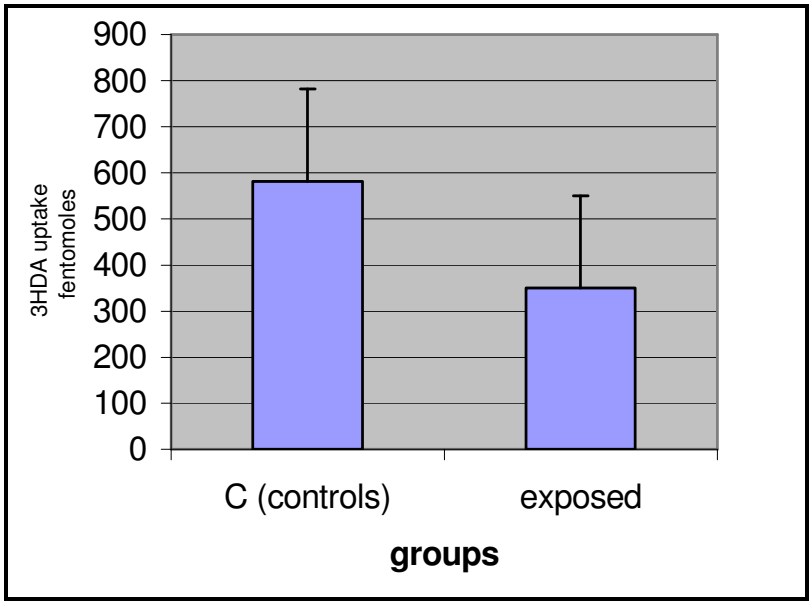

Fig. (5): The effect of lead acetate on H3DA uptake rates in synaptosomes isolated from the brain of controls and Pb-exposed rats. Results represent mean values ±SD of five experiments (the difference is significant, P<0.05).

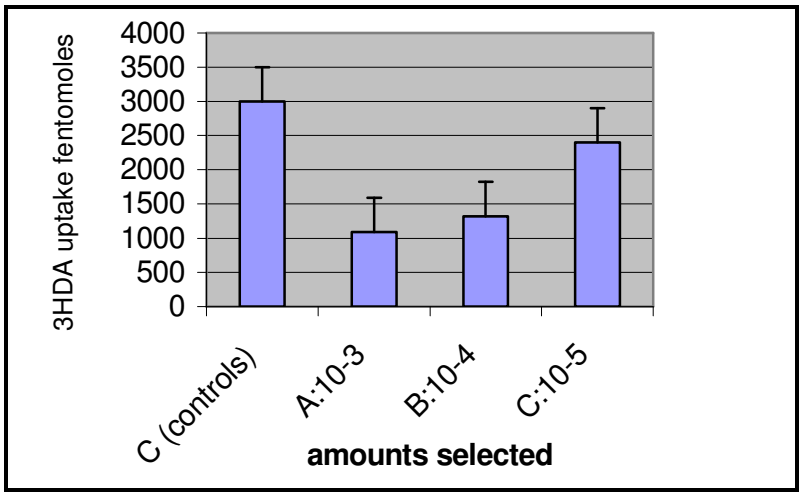

Fig. (6): The effect of lead acetate on H3DA uptake rates in synaptosomes isolated from the brain in vitro of controls and (amounts selected of lead acetate from 10^{-3} M to 10^{-5} M). Results represent mean values ± SD of five experiments.

DISCUSSION

Different authors have reported that the preferential site of the action of lead in the central nervous system is the glutaminergic system[5]. However the interaction of lead with other neurotransmitter systems has not been well studied. To this end we studied the effects of lead on the behavior of rat related mainly to the dopaminergic system[4,6]. The accumulated data confirm that there are toxic effects by lead on dopamine-dependent behavior[7]. The dopaminergic receptors (D1 and D2) have been implicated in the stereotypic behaviour[8,9,10]. Our findings suggest that lead induces a reduction in the catecholaminergic transmission, either by an inhibition of the synthesis of dopamine and its release to the synaptic site, or by an inhibition of the postsynaptic D2 receptors.

These findings are consistent with the work undertaken by others[11,12,2], which showed that intoxication with lead induces a reduction in the metabolites of dopamine (DOPAC, and homovanillic acid-H.V.A-) in the nigrostriatal and mesolimbic systems. Some of these researchers reported the presence of neurological disorders and an imbalance in the DA/Glu ratio in the limbic system[2]. Similarly, other researchers[13,1,14,15] observed a reduction in the conduction properties of the peripheral nervous system and activation of monoamino oxiadse.

This results in a reduction in the levels of dopamine at the synaptic level resulting in the reduction in dopaminergic transmission. Lead suppresses activity-associated Ca2+ - dependent release of acetylcholine, dopamine and amino acid neurotransmitters[16]. In particular, lead down- regulates dopamine, a neurotransmitter that plays an essential role in the inhibitory pathways of basal ganglia[17].

Stud undertaken by various authors[18,19,20]. We observed a modification in the gain of weight of the rats during this period of exposure to lead for about 90 days. Similarly, the results presented in table1 show 1000 ppm lead acetate administered by oral way for 30 days causes a very significant difference in treated rats, compared with the controls. These results show that the intoxication with lead affects the growth regulation. This is a reflect the influence of lead on the catecholaminergic and dopaminergic transmission.

Lead also has been shown to affect renal function and blood pressure. Some of the neurotoxic consequences to lead are: demyelinsation, inhibition of axonal activity in various cerebral structures (frontal cortex, striatum and hippocampus), convulsions, and memory disturbances[21,22]. Various authors showed that the NMDA (N-methyl-D-asparatate) system is targeted by lead. Lead blocks NMDA receptors and inhibits the flux of Ca[5,23]. Hhowever, the interaction of lead with other neurotransmitter systems such as dopaminergic, noradrenergic and serotonergic is likely as well[24]. Children who are independently mobile are at greatest neurological risk of chronic exposure to low or moderate levels of lead. From the time children are able to crawl until they enter school, they are at risk of ingesting lead-containing dust. While this sometimes is associated with pica and intentional ingestion of paint chips, lead poisoning often occurs without such behavior.

The long-term effect of lead exposure is maximal during the first 2 or 3 years of life, when the developing brain is in a critical formative stage[25].

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