

## ALTERATION OF PENTYLENETETRAZOL-INDUCED KINDLING PARAMETERS BY PRENATAL CHRONIC LEAD EXPOSURE IN RATS

ABBAS KEBRIYAEZADEH, REZA MASSIHAYEAKBAR, KAMBIZ SOLTANINEJAD

Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

### ABSTRACT

The effect of prenatal chronic lead exposure on pentylenetetrazol (PTZ)-induced kindling parameters (seizure index, seizure latency and seizure stage) in rats was studied. Adult female rats with a weight range of 140-180 g were selected and pretreated with lead acetate (0.05 %w/v) orally, 25 days prior to mating. The control group was given distilled water containing sodium acetate solution (0.05%w/v). After delivery, treatment was ceased, and after lactation, male neonates were separated from the females in both groups. After maturation of male rats, the PTZ-kindling was induced by daily intraperitoneally injection of PTZ (30 mg/kg). Kindling parameters in the control and treated groups were determined. The results indicated that animals with prenatal lead exposure have full kindling state with 9-19 ( $16.87 \pm 1.54$ ) injections, whereas this value for control group was 12-23 ( $18.62 \pm 1.48$ ) injections. The seizure latency for the treated group was lower ( $p < 0.05$ ) than the control ( $2.29 \pm 0.44$  min versus  $3.65 \pm 0.45$  min). The seizure severity (regarding to seizure index) was statistically higher in the treated group ( $p < 0.05$ ). The seizure stages were also different in the treated and control groups ( $p < 0.05$ ). The seizure frequency of first and second stages of kindling in the control group was higher than that of treated one ( $p < 0.05$ ). Also the seizure frequency in the third and fourth kindling stages of case group was higher than controls ( $p < 0.05$ ). It is concluded that prenatal lead exposure alters seizure susceptibility in rat PTZ-kindling model.

**Key words:** Lead, PTZ, Kindling, Prenatal, Rat

### INTRODUCTION

Lead (Pb) is one of the most important environmental pollutant and is known to be a potent neurotoxin that inducing neurological damage and behavioral disruptions in humans and experimental animals (1). It is now widely believed that the fetus and children are more at risk from lead exposure than in the adult (2). Young animals and human who have been exposed to lead can be categorized as having clinical or subclinical poisoning depending on symptoms and blood lead content (3). A neurological consequence of lead intoxication is the development of seizure activity (4). This toxic effect, which has been reported to occur in a high percentage of children with lead poisoning (4), is apparently related to a central toxic action. Also, the kindling is one the most suitable model for the studies on epilepsy (5). In this model, the signs of

the complex partial seizure are induced by repeatedly subconvulsive threshold of electrical or chemical stimulations (5). Therefore we decided to investigate the effects of prenatal chronic lead exposure on the pentylenetetrazol (PTZ)-induced kindling.

### MATERIALS AND METHODS

#### *Animals:*

Female albino rats (140-180g) were obtained from Razi institute (Karaj-Iran) and housed in separate cages with free access to food and water and maintained on a 12-h light/dark cycle at room temperature.

#### *Treatment:*

Adult female rats divided into two groups. Animals were received 0.05% w/v sodium acetate solution for 25 days prior to mating (control

group) and animals were received 0.05%w/v lead acetate solution in same period (treated group). Lead acetate ( $\text{CH}_3\text{COOH}$ )<sub>2</sub>  $\text{Pb}\cdot 2\text{H}_2\text{O}$  (Merck Co., Germany) and sodium acetate ( $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$ ) (Merck Co., Germany), were dissolved in distilled water and 0.5 ml HCl was added to one liter of lead solution for prevention of lead salts precipitation. Solutions were prepared every second day. After 25 days of treatment mating was performed and controlled by vaginal smear test in both groups. After delivery the pretreatment was ceased. Post-weaning, male neonates were separated from the female in the both groups. After maturation, the PTZ-kindling was performed on 8 adult male rats (210-260g) in case and control groups.

#### *PTZ-kindling:*

Pentylentetrazol (PTZ) (Sigma Chemical Co., USA) was injected in a dose of 30mg/kg intraperitoneally everyday. PTZ was dissolved in 0.9% isotonic saline solution to provide a volume of 2ml/kg for intraperitoneal(i.p.) injection(6). All injections were performed between 9a.m. and 1p.m. ; After PTZ injection the behavior of animals was observed for 30 min. The intensity of behavioral seizures was evaluated by use of five point scoring system, similar to that previous describe by Racine (7):

Stage 1: Mouth and facial movements (rhythmic jaw opening). Stage 2: Head nodding. Stage 3: Forearm clonus. Stage 4: Rearing. Stage 5: Rearing and falling (loss of righting reflex).

The animals were injected with PTZ until they reached the kindling criteria, the full kindle rats were animals showing five times stage 5 during five consecutive injections. Using the values obtained from single animals, the mean value of seizure index (SI) was calculated for each group. In this model seizure index shows seizure severity and calculated from this formula:

$$\text{SI} = \text{A} + 2\text{B} + 3\text{C} + 4\text{D} + 5\text{E} / \text{A} + \text{B} + \text{C} + \text{D} + \text{E}$$

A, B, C, D and E represent the mean values of movements in the first, second, third, fourth and fifth stages of kindling(8).

#### *Statistics:*

Statistical analysis was carried out using the SPSS software. Kolmogorov-Smirnov test was conducted for normal distribution of individual data. All data were normally distributed and subsequently the paired t-test was performed for

seizure indexes, seizure stages, and seizure latency of each groups. Differences with a P value of less than 0.05 being considered significant. All values are expressed as means±SE.

## RESULTS

The criterion of kindled seizures in both groups of rats was manifested after different number of single PTZ injection. Animals with prenatal lead exposure showed the full kindling state with 9-19 ( $16.87 \pm 1.54$ ) injections whereas for the control group it was with 12-23 ( $18.62 \pm 1.48$ ) injections. The seizure latency for the case was lower than the control significantly ( $2.59 \pm 0.44$  min. versus  $3.65 \pm 0.45$  min)( $P < 0.05$ )(Fig. 1).

Seizure severity (regarding to seizure index) was statistically higher in the case group. Maximum and minimum of seizure index in lead treated rats were ( $3.56-1.6$ ) and for control group was ( $2.28-0$ )(Fig.2). Case group reached the first peak of seizure index after ( $16.87 \pm 1.54$ ) injection, but the peak of seizure index for control group was reached after ( $18.62 \pm 1.48$ ) injection. Seizure stages were different in cases and controls.

Seizure stages of treated animals were significantly higher than that of controls ( $p < 0.05$ ) (Fig.3). Seizure frequency of the first and second stages of kindling was also lower in the treated group (Table 1)( $p < 0.05$ ). Seizure frequency in the third and fourth kindling of the treated group was higher than the control one ( $p < 0.05$ )(Table 1).

## DISCUSSION

The present data indicate that seizure severity (considering seizure index and seizure stages) were different in the two groups. Seizure index of the treated group was higher and seizure latency was lower when compared to controls. Parameters which provided a measure of seizure severity (e.g. seizure index) were enhanced in animals exposed to lead, additionally, the occasional occurrence of biphasic seizure in lead- exposed but not in control rats may also be indicated of a more seizure state in rats exposed to lead starting in neonatal life. Lead enhances those events which are involved in the intensity and spread of a given seizure episode. Clinical and experimental data indicated that lead intoxication in early life has more profound neurotoxicological consequences than intoxication in adulthood (4).

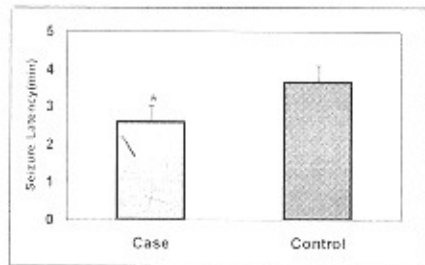


Fig.1. The effect of prenatal lead exposure on seizure latency in case and control group (n=8. \*P<0.05).

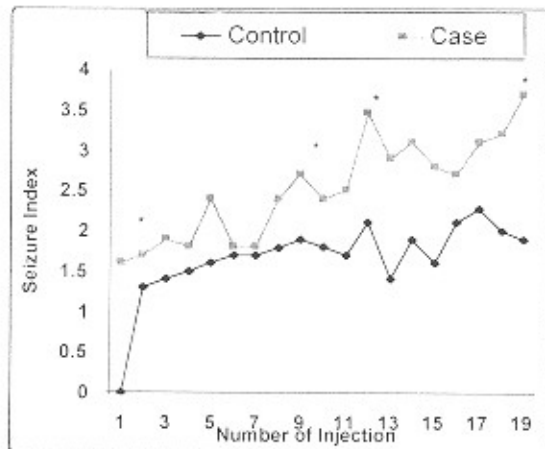


Fig.2. Comparison of seizure indexes in case and control rats. Animals were injected i.p. with PTZ (30 mg/kg), every 24 hours. (\*P< 0.05, n=8).

The interaction between lead intoxication in early life and other models of seizure activity has been studied previously, for example, investigators found that neonatal lead intoxication enhanced both the susceptibility and severity of maximal electroshock-induced seizure at various stages of development (9). Similarly, some investigators found that early exposure to lead produced an increase in the sensitivity to the behavioral effects of a variety of chemical convulsants (10,11). Studies suggested that lead is exerting its effects through noncompetitive interactions with presynaptic GABA transport mechanisms and/or lead exposure is associated with a decrease in the number of GABA transporting site, possibly as a

result of specific destruction of GABA releasing neurons (10).

On the other hands, the site of action of PTZ has been reported the cortex, reticular formation or mammillary bodies. The mechanism of action of PTZ surely involves GABA metabolism. This effect is probably at the synapses, where PTZ acts at the GABA receptor complex in a way that decreases the inhibitory capacity of GABA (5). Further treatment with anticonvulsant drugs, such as the GABA agonist, increase the number of stimulations needed to induced fully kindled seizures (12).

Although other contributory mechanisms may be operational (13,14). Studies have shown that lead is a potent and selective non-competitive antagonist of the N-Methyl-D-Aspartate (NMDA) receptor complex which an excitatory amino acid receptor subtype that plays an important role in brain development. The inhibitory effects of lead on the NMDA receptor complex were shown to be age-dependent, with immature brain tissue being more sensitive than mature tissue (11). These finding are important because the activation of this glutamate receptor subtype has been implicated in the regulation of neuronal morphology and synaptogenesis. Studies on the pathogenesis of kindled seizure have implicated a number of neurochemical alteration in this model of epileptogenesis (15). One group of neurotransmitters which has been repeatedly implicated in the regulation of kindled seizure as well as in other seizure models (14,16), is the catecholamines, particularly norepinephrine. According to this hypothesis central noradrenergic mechanisms exert a tonic inhibitory influence against seizure activity which is disrupted during the kindling process(16). From this view point, prenatal lead exposure, may alter the central neurotransmitter systems that affects epilepsy (e.g. GABAergic system) and increases seizure susceptibility in PTZ-kindling model.

Table 1. The number of movements in kindling stages in rats

Number of Movements	Control	Lead-treated
Stage 1	62 ± 1.34	45 ± 2.01*
Stage 2	55 ± 1.53	24 ± 0.82*
Stage 3	1 ± 0.13	5 ± 0.42*
Stage 4	68 ± 4.46	121 ± 4.43*

Each point is the mean±SEM of 8 animals. \*P<0.05 different from control group.

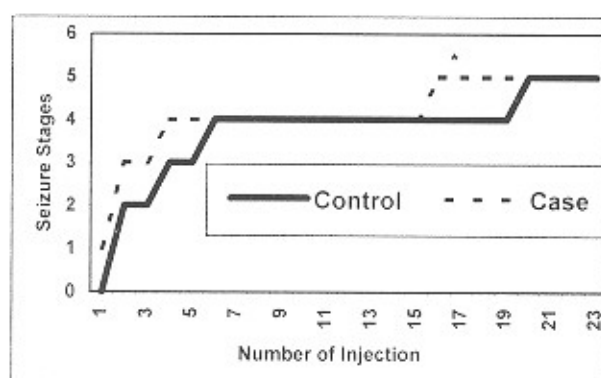


Fig.3. Seizure stages in case and control rats (n=8, P<0.05)

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