

## ALTERATION OF PENTYLENETETRAZOL- INDUCED KINDLING PARAMETERS BY PRETREATMENT OF RATS WITH PROPYLTHIOURACIL AND LEAD ACETATE

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### ABSTRACT

Lead is known to be a potent neurotoxin that can cause seizure activity in humans and animals. Also, epileptic type convulsion is a known complication of hypothyroidism. This study was performed to determine the effect of propylthiouracil (PTU)-induced hypothyroidism and chronic lead exposure on pentylenetetrazol (PTZ)-induced kindling parameters. The test was carried out by using male albino rats weighing 180-230 g which were divided into four groups as follows:

Group 1: rats which received distilled water for 25 days (control group);

Group 2: rats pretreated with an orally administered lead acetate solution (0.05%) for 25 days;

Group 3: rats rendered hypothyroid with PTU solution (0.1%W/V) for 25 days; and

Group 4: rats co-treated with lead acetate solution (0.05%W/V) and PTU solution (0.1%W/V) for 25 days.

On the 26th day of the experiment PTZ (ip, 30 mg/kg) was injected once a day in order to induce seizure behaviors.

Kindling parameters such as seizure latency, index, stage and frequency were evaluated. In animals, both lead acetate (0.05% W/V) and PTU (0.1%W/V) pretreatment caused significant alterations in kindling parameters separately. But in animals pretreated with a combination of lead acetate and PTU, potentiation of kindling parameters was not observed compared to groups one and two. In conclusion, lead acetate and PTU may affect kindling parameters by different mechanisms.

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## INTRODUCTION

Lead is known to be a potent neurotoxin inducing neurological damage and behavioral disruptions in humans and experimental animals, with developing individuals being particularly susceptible.<sup>1</sup> One neurological consequence of lead intoxication is the development of seizure activity.<sup>2-5</sup> This toxic effect, which has been reported to occur in a high percentage of children with lead poisoning, is apparently related to a central toxic action.<sup>1</sup> On the other hand, relations between hypothyroidism and neurological function are well recognized.<sup>6</sup> For example, in elderly hypothyroids, mental change, confusion, paranoia, depression and dementia are often attributed,<sup>7</sup> and autistic disorders have been observed in hypothyroid children.<sup>8</sup>

There is also evidence that epileptic-type convulsion is a complication of severe hypothyroidism.<sup>6,9,10</sup> As long ago as 1922 it was shown that there is an increased susceptibility of rabbits and cats to induced convulsion following thyroidectomy.<sup>11,12</sup> Later, administration of synthetic thyroxine was shown to reduce the incidence of photically induced epileptic responses in the baboon *Papio papio*.<sup>13</sup>

In genetically epilepsy prone rat (GEPR-9) model, the critical impact of neonatal hypothyroidism on brain function coupled with the development of audiogenic seizure susceptibility during the third week after birth, suggested that neonatal hypothyroidism could be an etiological factor in the development of the seizure-state of GEPR-9 rats.<sup>14</sup>

There are many conditions in which hypothyroidism may accompany lead exposure throughout life. Therefore it is important to examine the combination effects of hypothyroidism and lead exposure on cases suffering from epilepsy. With respect to these reports, we were interested in determining the effects of PTU-induced hypothyroidism on PTZ-induced kindling parameters in chronic lead pretreated rats.

## MATERIALS AND METHODS

## Animals

24 male albino rats weighing 180-230 g 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/12-h dark schedule at a temperature of  $22 \pm 2^\circ\text{C}$ .

## Chemicals

Lead acetate [ $(\text{CH}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$  (Merck, Germany)] was dissolved in distilled water. In order to prevent the precipitation of lead salts, 0.5 mL of HCl (1N) was added to 1 liter of distilled water. PTU (Sigma Chemical Co., UK) was dissolved in distilled water. PTZ (Sigma Chemical Co., UK) was dissolved in 0.9% isotonic saline solution to provide a volume of 2 mL/kg for intraperitoneal (i.p.) injection, as described previously.<sup>15</sup>

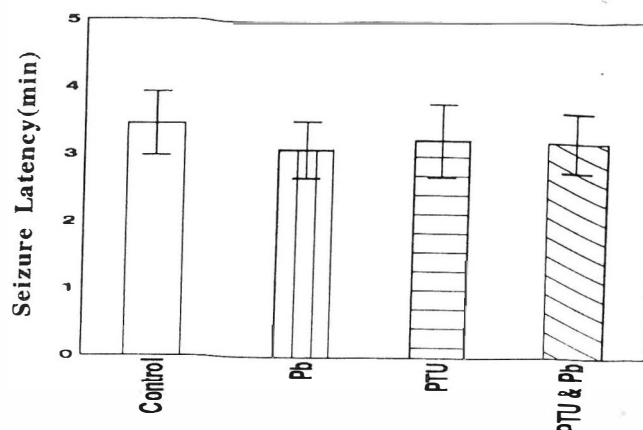


Fig. 1. Seizure latency in full kindled treated and control rats ( $n=6$ ,  $p<0.05$ ).

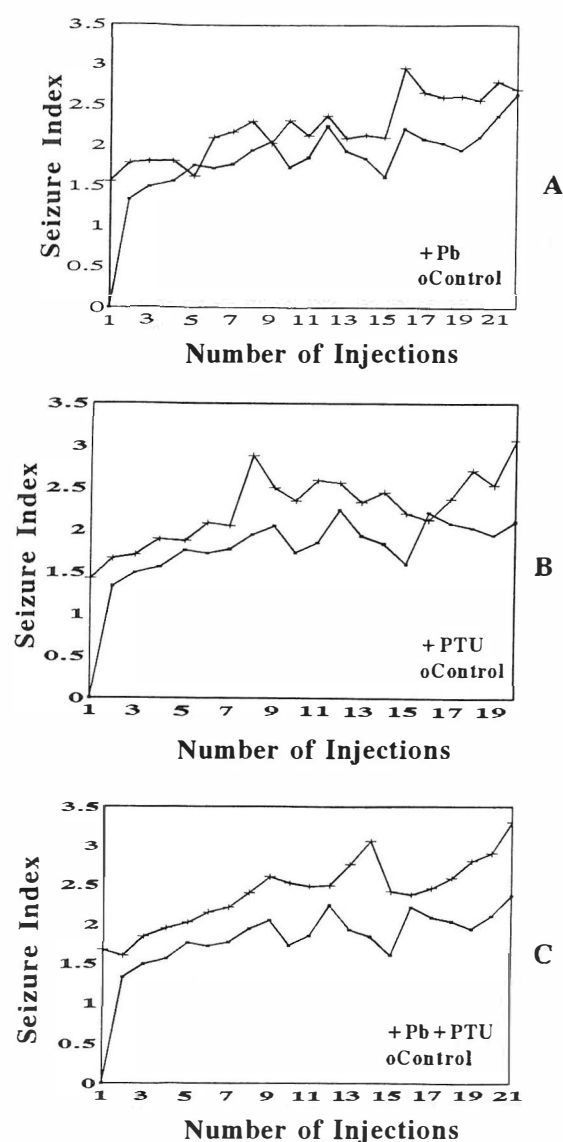


Fig. 2-A, B, C. Seizure index in treated (+) and control (●) groups after daily injection of PTZ (30 mg/kg, i.p.) ( $n=6$ ,  $p<0.05$ ).

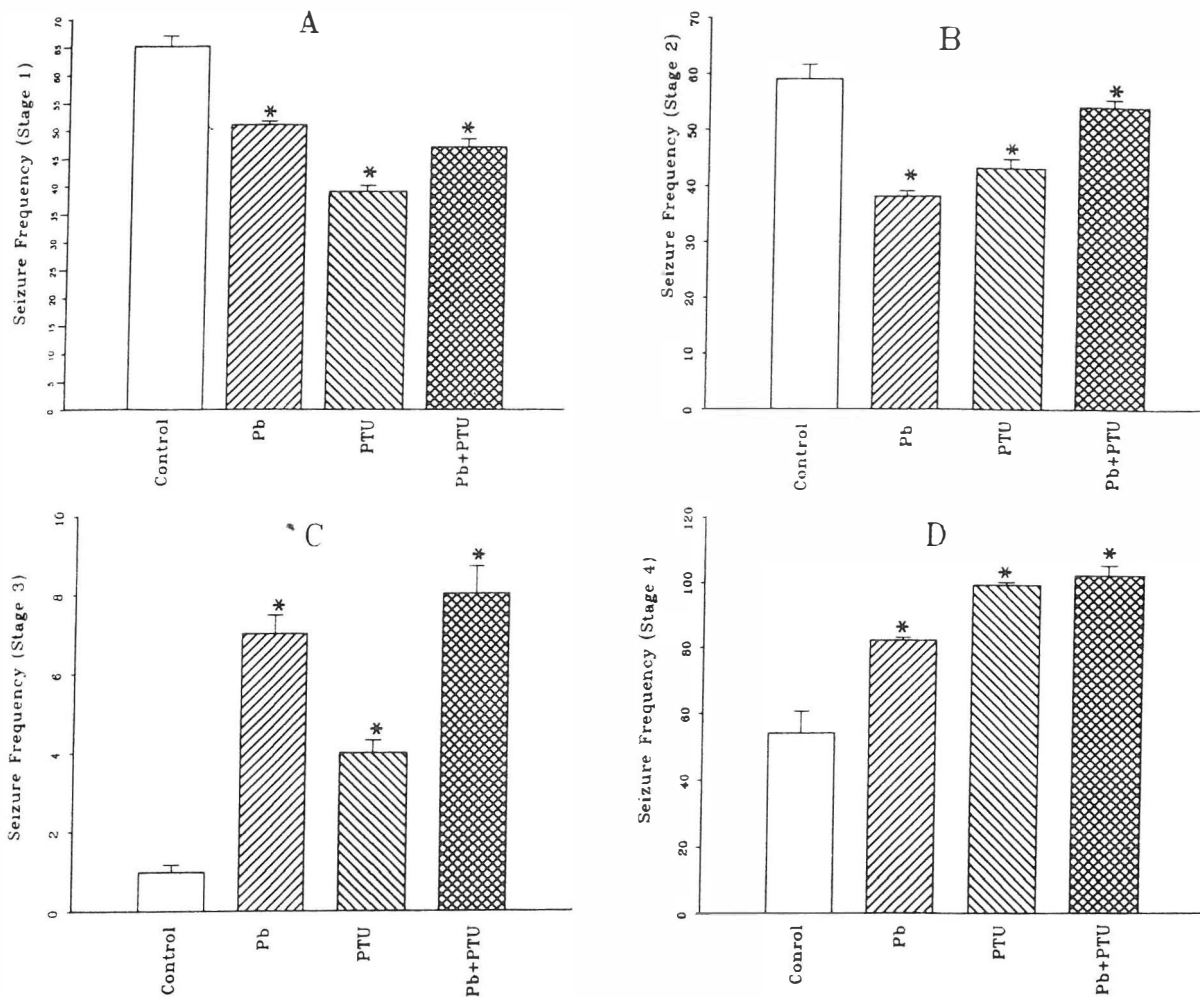


Fig. 3-A, B, C, D. Seizure frequency of kindling stages in treated rats in comparison with controls ( $n=6, p<0.05$ ). Values are means  $\pm$  S.E.

#### Treatment schedules

Animals were divided into four groups as follows:

Group 1: Animals receiving only distilled water (acidified) for 25 days (control group,  $n=6$ ).

Group 2: Animals receiving lead acetate solution (0.05%) for 25 days ( $n=6$ ).

Group 3: Animals receiving PTU solution (0.1%W/V) for 25 days, as drinking water ( $n=6$ ).

Group 4: Animals receiving water containing lead acetate (0.05%) and PTU (0.1%) for 25 days ( $n=6$ ).

After 25 days, treatment was ceased and on the 26th day of the experiment PTZ-kindling was performed on animals in all groups.

#### PTZ-kindling

PTZ was injected i.p. in a dose of 30 mg/kg every day. All injections were made between 8 a.m. and 2 p.m. After PTZ injection the behavior of animals was observed for 30 min. The intensity of seizures was evaluated by use of a five

point scoring system similar to that previously described;<sup>16</sup> 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 criterion (five times stage 5 during five consecutive injections). By using the values obtained from each animal, the mean value of seizure index (SI) was calculated for each group. In this model SI shows seizure severity and is calculated from this formula:

$$SI = A + 2B + 3C + 4D + 5E / A + B + C + D + E$$

A, B, C, D & E indicate the means of movements in kindling stages 1, 2, 3, 4 & 5 respectively.

#### Statistical analysis

Statistical analysis was carried out by computer using the SPSS statistical program. The Kolmogorov-Smirnov test was conducted for normal distribution of individual data. All data were normally distributed, and subsequently the

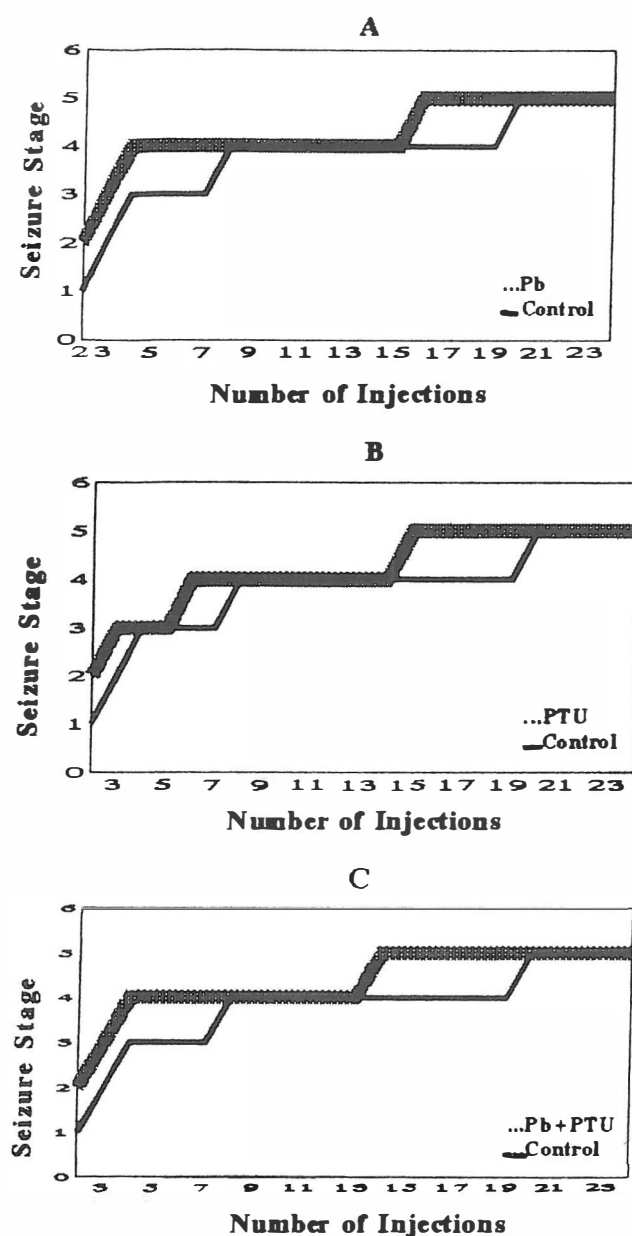


Fig. 4-A, B, C. Seizure stages in treated rats (...) in comparison with controls (-) after a course of PTZ-kindling ( $n=6$ ,  $p<0.05$ ).

paired t-test was performed for seizure indices and seizure stages and the t-test was also performed for the seizure latency of each group. Differences with a P value of less than 0.05 were considered significant.

## RESULTS

### Seizure latency

Results showed that seizure latency had no significant difference in all groups ( $p<0.05$ ) (Fig. 1).

### Seizure index (SI)

Maximum and minimum values of SI were (2.66, 0) for

group 1, (2.96, 1.55) for group 2, (3.06, 1.42) for group 3 and (3.3, 1.67) for group 4 (Fig. 2-A, B, C). Lead pretreatment together with PTU-induced hypothyroidism increased the SI of the 4th group when compared with controls ( $p<0.05$ ) (Fig. 2-C). There was no significant difference concerning the seizure index between the lead pretreated and PTU induced hypothyroid rats.

### Seizure frequency

Seizure frequency in the 1st and 2nd phases of kindling was low in the rats of all treated groups, after reaching the full kindling state, compared to the control group ( $p<0.05$ ) (Fig. 3-A, B), but seizure frequency in the 3rd and 4th phases of kindling was high compared to controls ( $p<0.05$ ) (Figs. 3-C, D).

### Seizure stages

Significant differences in seizure stages between the control and treated groups were seen ( $p<0.05$ ) (Fig. 4-A, B, C), but among the 2nd, 3rd and 4th groups, no significant differences in seizure stages were observed.

## DISCUSSION

The present data demonstrate that chronic lead pretreatment or PTU-induced hypothyroidism increases the severity of kindling seizures in PTZ-kindled rats (according to seizure stages and seizure indices), but lead pretreatment together with hypothyroidism has no synergistic effect on PTZ-kindling parameters. These effects might actually be related to the influence of lead and hypothyroidism on the kindling state.

The nervous system is a major site of lead toxicity.<sup>2,3</sup> The mechanisms of lead neurotoxicity are not fully understood.

Several studies have shown that lead exerts its neurotoxic effects by two distinct mechanisms:

- 1) As a neurodevelopmental toxicant interfering with the differentiation of the central nervous system (CNS); and
- 2) as a neuropharmacological toxicant interfering with the ionic mechanisms of neurotransmission.<sup>17</sup>

There are contradictory reports regarding the possible roles of the central neurotransmitter system in the development of low level lead neurotoxicity, because an increase, decrease or no change in the functional capacity of the GABAergic, cholinergic, adrenergic and glutamatergic system in the CNS have all been reported.<sup>18-21</sup> Epilepsy-type seizures occur in severe hypothyroidism and the pathogenesis of seizure remains unclear. It was suggested that impaired liver glycogenolysis and reduced adrenocortical function caused by an absence of thyroxine produce mild hypoglycemia. Alternatively, it could be that increased interstitial and intracellular fluids and the reduction in plasma volume seen in patients with myxedema, along with

alterations in the blood brain barrier, result in increased cerebrospinal fluid and cerebral edema which may cause the fits. Animal studies suggest that seizure potential varies with thyroid function independent of metabolic rate.<sup>5</sup>

In our study, chronic lead exposure caused an increment of seizure severity compared to controls. This effect is probably related to GABAergic neuron abnormalities. Lead potentially affects GABAergic neurotransmission and decreases the presynaptic function of GABAergic neurons in exposed rats,<sup>22</sup> and PTZ also acts at the GABA receptor complex in a way that decreases the inhibitory capacity of GABA.<sup>19</sup> Our results in lead treated rats were generally similar to those described previously by other investigators.<sup>23-27</sup>

In contrast, seizure latency in lead treated rats does not differ from the control group significantly. Therefore it appears that lead may have different effects on kindled seizures, enhancing those events which are involved in the intensity and spread of a given seizure episode while having little or no effect on factors which are responsible for initiation of seizure activity. From this point of view, animals intoxicated with lead at birth appear to have no difference from those intoxicated as adults.<sup>28</sup>

PTU-induced hypothyroidism increased the seizure severity in treated rats. This is probably due to a direct effect of thyroid hormones or rather its lack of membrane excitability; nevertheless, the major mechanism of this event is unclear.<sup>10</sup> Our results also revealed no synergism between PTU-induced hypothyroidism and chronic lead pretreatment on PTZ-induced kindling parameters. Some studies on the pathogenesis of kindled seizures have indicated that a number of neurochemical alterations accompany this model of epileptogenesis.<sup>26</sup> One group of neurotransmitters which has been repeatedly implicated in the regulation of kindled seizures as well as in other seizure models<sup>29</sup> is the catecholamines, particularly norepinephrine. *In vivo*, lead increases norepinephrine concentration in the whole forebrain in mice and rats.<sup>22</sup> In contrast, in rats which were made hypothyroid, lower brain monoamine levels were reported.<sup>14</sup> As the result, synergism or antagonism was not found between chronic lead pretreatment and PTU-induced hypothyroidism on PTZ-induced kindling parameters. Further studies to find the exact mechanism of the effect of lead acetate or hypothyroidism on kindling parameters are necessary.

## REFERENCES

- Alfano DP, Petit TL, LeBoutiller JC: Development and plasticity of the hippocampal cholinergic system in normal and early lead exposed rats. *Dev Brain Res* 10: 117-124, 1983.
- Allen JR, McWay PT, Suomi SJ: Pathobiological and behavioral effects of lead intoxication in the infant rhesus monkey. *Environ Health Perspect* 7: 239, 1974.
- Perlstein MA, Attala R: Neurologic sequelae of plumbism in children. *Clin Pediatr (Phila.)* 5: 292, 1966.
- Silbergeld EK: Mechanisms of lead neurotoxicity: looking beyond the lamppost. *FASEB J* 6: 3201-3206, 1992.
- Silbergeld EK, Miller LP, Kennedy S, Eng N: Lead, GABA and seizure: effects of subencephalopathic lead exposure on seizure sensitivity and GABAergic function. *Environ Res* 19: 371-382, 1979.
- Rowell NP, Clarke SW: Myxedema presenting as epilepsy. *Postgrad Med J* 60: 605-606, 1984.
- Braunstein GD, Friedman T, Herman-Bonert V, Peters A: Endocrine Disease. In: Andreoli TE, Bennett JC, Carpenter CCJ, Plum F (eds.), *Cecil Essentials of Medicine*. Philadelphia: W.B. Saunders Co., pp. 487-496, 1997.
- Gillberg C, Gillberg CH, Kopp S: Hypothyroidism and autism spectrum disorder. *J Child Psychol Psychiat* 33 (3): 531-542, 1992.
- Evans EC: Neurological complications of myxedema convulsions. *Ann Intern Med* 25: 434-444, 1960.
- Bryce GM, Poyner F: Myxedema presenting with seizures. *Postgrad Med J* 48: 35-36, 1992.
- Uyematsu S, Cobb S: Preliminary report on experimental convulsions. *Arch Neurol Psychiatry* 7: 660, 1922.
- Elsberg C, Stookey B: Studies on epilepsy. *Arch Neurol Psychiatry* 9: 613, 1923.
- Serbanscu T, Balzamo E: The influence of thyroxine in photosensitive *Papio papio* baboons. *Electroencephalogr Clin Neurophysiol* 36: 253-258, 1974.
- Mills SA, Savage DD: Evidence of hypothyroidism in the genetically epilepsy-prone rat. *Epilepsy Res* 2 (2): 102-10, 1988.
- Diehl RG, Smialowski A, Gottwo T: Development and persistence of kindled seizures after repeated injections of pentylenetetrazol in rats and guinea pigs. *Epilepsia* 25 (4): 506-510, 1984.
- Racine RG: Modification of seizure activity by electrical stimulation: motor seizure. *Electroenceph Clin Neurophysiol* 32: 281-294, 1972.
- Moor MR, McIntosh MJ, Bushnell WR: The neurotoxicity of lead. *Neurotoxicology* 7: 541-556, 1986.
- Jett DA, Guilarte TR: Developmental lead exposure alters N-methyl-D-aspartate and muscarinic cholinergic receptors in the rat hippocampus. An autoradiographic study. *Neurotoxicology* 16 (1): 7-18, 1995.
- McCandless DW, Finesmith RB: Chemically induced models of seizure. In: Boulton A, Baker G, Butterworth R (eds.), *Neuromethods: Animal Models of Neurological Disease*. Vol. 22, New York: The Humana Press, pp. 131-151, 1992.
- Ramsy PB, Krigwan MR, Morell P: Developmental studies of the uptake of choline, GABA and dopamine by crude synaptosomal preparations after *in vivo* or *in vitro* lead treatments. *Brain Res* 187: 383-402, 1980.
- Cory-slechta DA, Pokora MJ: Lead-induced change in

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- muscarinic cholinergic sensitivity. *Neurotoxicology* 16 (2): 337-348, 1995.
22. Silbergeld EK, Hruska RE: Neurochemical investigation of low level lead exposure. In: Needlman HL, (ed.), *Low Level Lead Exposure: The Clinical Implication of Current Research*. New York: Raven Press, pp. 135-156, 1980.
23. Fox DA, Overman SR, Woolly DE: Neurobehavioral ontogeny of neonatally lead-exposed rats. 2. Maximal electroshock seizures in developing adult rats. *Neurotoxicology* 1: 149-170, 1979.
24. Joy RM, Albertson TE, Stark LG: An analysis of the actions of progabid, a specific GABA receptor agonist, on kindling and kindled seizures. *Exp Neurol* 83: 144, 1984.
25. Peterson SL, Albertson TE: Neurotransmitter and neuromodulator function in the kindled seizure state. *Prog Neurobiol* 19: 237, 1982.
26. Schwark WS, Haluska M, Powel K, Blackshear P: Lead intoxication and the amygdaloid kindling model of epileptogenesis in the adult rat. *Neurobehav Toxicol Teratol* 5: 325, 1983.
27. Shih TM, Hanin I: Chronic lead exposure in immature animals. Neurochemical correlates. *Life Sci* 23: 628-637, 1980.
28. Schwark WS, Haluska M, Blackshear P, Magana T: Lifetime lead intoxication: influence on the amygdaloid kindling model of epileptogenesis. *Toxicology* 36: 49-60, 1985.
29. Maynert EM, Marczyński TJ, Browning RA: The role of the neurotransmitters in epilepsy. *Adv Neurol* 19: 79, 1979.