

Preliminary Report

Effects of Toxic Metals on the Onset and Duration of Pentobarbitone-induced Sleep

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ABSTRACT

The chronic and acute manifestations and behavioral deviations on exposure at low dose levels to toxic metals are known. In relevant occupations, exposure to toxic metals by inhalation, dermal absorption and ingestion cause behavioral changes. By cumulative action, they lead to neurotoxicity and influence circadian rhythm and sleep patterns.

Objectives: To determine what influence toxic metals (lead, manganese, cadmium and zinc) have on the onset and duration of sleep, both before and after administration of pentobarbitone (PB).

Methods: Heavy metals (lead 65 mg/kg; manganese 2 mg/kg; cadmium 3 mg/kg; and zinc 2mg/kg) *ip*/single dose in 0.85% NaCl saline were administered 60 minutes before and 30 minutes after pentobarbitone treatment (30 mg/kg *ip*) in rats. The onset and duration of sleep by righting reflex in controls (saline *ip*) and metal-treated animals were recorded. Statistical analysis was done by employing students 't' test.

Results: Control rats (n = 5) receiving PB slept 217 ± 72.8 min (M ± SD) with a refractory period of 27 ± 12.5 min.

The pre-PB, metal-treated rats (n = 5/per metal) showed a delay in the onset of sleep by 110 ± 70.1, 36 ± 35.6, 43 ± 9.5 and 34 ± 11.7 minutes and the duration of sleep was 134 ± 34.5, 157 ± 76.9, 224 ± 53.1 and 161 ± 28.9 minutes of manganese, zinc, cadmium and lead, respectively (p < 0.01). The post-PB metal-treated rats (n = 5/per metal) slept 268 ± 74.1 minutes with cadmium and 213 ± 95.6, 197 ± 34.2 and 203 ± 41.9 minutes (M ± SD) with manganese, zinc and lead, respectively. All the metals dilated the onset of PB sleep whereas total sleep, except cadmium, reduced the sleep duration. In respect of metal treated post-PB in rats, cadmium enhanced (p < 0.01) sleep duration more than the other metals.

Conclusions: The pre-PB metals enhanced the onset of sleep, more with manganese. All metals, except cadmium, reduced the sleep duration. Metals had no effect after post-PB treatment except cadmium, which potentiated the PB effect. The paradoxical effect of metals before and after PB treatment indicates a delayed entry of PB across the blood-brain barrier and, during the refractory period, its partial degradation.

KEYWORDS: metals, pentobarbitone, rats, sleep

INTRODUCTION

Behavioral changes indicate the adverse effects of chemical^[1] or physical^[2] agents on intact organisms. It serves as a useful tool for measuring neurotoxicity^[3]. Moreover, changes in the pattern of electroencephalogram (EEG) elicited by stimuli producing arousal^[4], light^[5], sound^[6], electrical stimulation or produced by sleep^[7], will most probably enhance its usefulness in the study of neurotoxicity. Chronic and acute manifestation and behavioral deviations^[8] on exposure at low dose levels to trace metals are known. Exposure to toxic metals, in relevant occupations, by inhalation, dermal absorption and ingestion cause behavioral changes by cumulative action and they lead to neurotoxicity^[9]. Sleep is an integral phase of circadian rhythm in mammals. Inorganic lead (Pb⁺⁺) can damage both the central nervous system

(CNS) and peripheral nerves and cause disturbed memory, and encephalopathy^[10]. Manganese (Mn⁺⁺)^[11] causes earlier functional changes in basal ganglia which produce a Parkinsonian-type symptomatology. Similarly, zinc (Zn⁺⁺) and cadmium (Cd⁺⁺) are implicated with CNS effects either in the depression or excitation. The aim and objectives of the present study is to determine the sub-acute effects of trace metal on pre- and post-pentobarbitone (PB) treatment in rats by assessing the PB-sleeping time and the onset of sleep.

MATERIALS AND METHODS

Experiments were carried out on inbred Charles Foster male rats, weighing between 400-500g. Animals were maintained under constant conditions of light and temperature and were divided into three groups. The control group

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received *ip* saline (0.9% NaCl). The experimental group-I was treated with trace metals 60 min before PB (30 mg/Kg). The experimental group-II was treated 30 min. after PB. Animals were fed a staple diet (wheat flour 70%; bengal gram 20%; fish-meal 5%; dried yeast powder 4%; shark liver oil 1%) during the period of study. Latency and duration of PB sleep were calculated by measuring the time for loss (latency) and resumption (duration) of righting reflexes. Righting reflexes were tested continuously from the moment of *ip* PB and timed with a timer. Any failure of the animal to right itself within five seconds was considered as a loss of righting reflex^[12].

In experimental I, rats were pre-PB *ip* treated with the trace metals at sub-lethal doses^[13] of lead 65 mg/Kg, manganese 2 mg/Kg, cadmium 3 mg/Kg and zinc 2 mg/Kg and the latency period and duration of sleep were measured. In experiment II, rats were given *ip* PB and, 30 min later, the same doses of trace metals *ip* as were given to the experimental I group were injected. The duration of sleep was then measured. Students' 't' test was employed in the data analysis ($M \pm SD$)^[14].

Chemicals: The following metallic salts were used in this study: Zinc as $ZnSO_4 \cdot 7H_2O$ (Sarabhai Chemicals, India); Manganese as $MnCl_2 \cdot 4H_2O$ (Loba Chemicals, India); Lead as $Pb(CH_3COO)_2 \cdot 3H_2O$ (Glaxo, India); Cadmium as $CdCl_2$ (Loba Chemicals, India); Sodium chloride (Glaxo, India); Sodium pentobarbitone (May & Baker, Germany). All the chemicals were of analytical grade. The solutions were made in ion-free triple distilled water.

RESULTS

In control rats receiving saline PB, sleeping time was found to be 217 ± 72.8 min, whereas in the experimental group I ($n = 5$ -- 60 min pre-PB treated with manganese, zinc, cadmium and lead) the sleeping duration (time) was 134 ± 34.5 , 157 ± 76.9 , 224 ± 53.1 and 161 ± 28.9 min, respectively (Fig. 1). Thus, there was a decrease in sleeping time with manganese, zinc and lead ($p < 0.01$) but an insignificant change in sleep with cadmium. In experimental group-II ($n = 5$ -- 30 min after PB, metal treated rats) there was an increase ($p < 0.01$) in sleeping period with cadmium (268 ± 74.1 min) whereas with manganese, zinc and lead, the PB-sleep period was 213 ± 95.6 , 197 ± 34.2 and 203 ± 41.9 min, respectively (Fig. 1).

The latency period was 27 ± 12.5 in the controls. In experimental group-I, the latency period was 110 ± 70.1 , 36 ± 35.6 , 43 ± 9.5 and 34 ± 11.7 min, respectively for manganese, zinc, cadmium and lead. There was a significant increase ($p < 0.01$) in

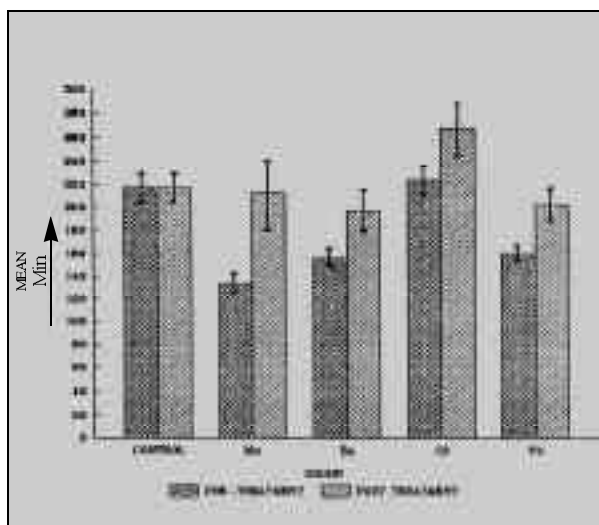


Fig. 1: Comparative metallic hypnoeffects in rats (sleeping period) [Mean \pm SD]

latency period with manganese in the experimental group I. Cadmium showed a mild rise in PB-sleeping time in experimental group I and a prolonged sleep duration of rats in the experimental group II. Our data showed a significant increase in the sleeping period of PB-treated rats only with cadmium but not with other metals.

DISCUSSION

Toxicity by metal alters sleep patterns, results in sleep deprivation and deficits in learning. The sleep mechanism and their significance in metabolism, adaptation, health recovery, learning, recoupage of energy and essential metabolite human and animal behavior reflexes as well as the in-memory process have been extensively explored. In the occupational sector, sleep is the most sensitive behavioral unit and is liable for change due to exposure to biological, physical and chemical agents, which may elicit psychological response indicating health impairment. PB-sleep has been a handy conventional tool to study the influence of chemicals^[3]. In our study, the 60 min before pre-PB treated rats showed decreases in PB-sleeping time with manganese, zinc and lead, and a mild increase of sleep with cadmium. While in the experimental group II, 30 min post-PB showed an increase in sleeping period with cadmium but no change in respect of manganese, zinc and lead in comparison with controls.

There was significant increase in sleeping period of PB rats treated with cadmium than with other metals such as manganese, zinc and lead. In the PB sleep periods, the cadmium pretreated group showed a mild rise and manganese showed maximum attenuation of sleep duration. Heavy metals influenced the latency period in pre-PB treated rats. The sleeping period in the

experimental group II showed increase in sleeping time with cadmium treated rats when compared to manganese and other metals. The barbiturate - induced sleep, in turn, is due to inhibition of the release and turnover of acetylcholine in the cortex and brain^[4]. Experimental group I rats showed more decrease in sleeping time with manganese than with other metals.

Chronic exposure to lead (0.2 mg/ml and 0.5 mg/ml) in drinking water for 90 days in rats did not show significant alterations during its pre-and post-PB administration. The chronic lead exposure induced enhanced adrenergic and diminished cholinergic functions^[5] and interfered with dopaminergic turnover^[6] in the central nervous system^[7]. The present data is limited to record the changes in the duration of sleep when administered before and after PB in rats exposed to acute dose of LD50 of lead. Kadiiska et al.,^[8] reported decreased hexobarbital sleeping time in rats receiving 100 mg zinc sulphate/kg/day. This physiological response suggested an induction of microsomal enzymes^[15]. Our observations with zinc at the dose of 2mg/kg (zinc sulphate) also decreased the effects by other metals^[9]. Excessive manganese ingestion could lead to learning or behavioral impairment in children. In our study, manganese in the dose of 2mg/kg *ip* reduced sleeping time in pre-PB treated rats while there was no significant change in sleeping time of post-PB treated rats. Cadmium showed mild alteration in pre-PB treated rats but post-PB treated group showed a steep increase in sleeping time which may be due to its effect on liver (i.e. hepatotoxicity^[10]) or the release of brain acetylcholine^[11]. The paradoxical effect of metals before and after PB-treatment indicates a delayed entry of PB across the blood-brain barrier and, during this refractory period, its partial degradation. Metals had no effect after post-PB treatment except cadmium which potentiated the PB effect and, thus, it is unlikely to be dependent on brain cholinergic system^[16].

CONCLUSION

All the pre-PB trace metals enhanced the onset of sleep; manganese to the greatest degree. All metals, except cadmium, reduced the sleep duration. Metals had no effect after post-PB treatment, except cadmium which potentiated the PB effect. The paradoxical effect of metals before and after PB treatment indicates a delayed entry of PB across the blood-brain barrier and, during the refractory period, its partial degradation.

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