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Decrease in brain serotonin level and short term memory loss in mice: a preliminary study

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Abstract

Most of the information on the effects of benzene center around its hematotoxic and genotoxic effects. However, its effect on central neurotransmitters is inconclusive in terms of cognitive behavior of the host. The present results showed for the first time that chronic exposure to benzene, in drinking water, significantly inhibited serotonin (5-hydroxytryptamine (5-HT)) level in serotonergic neuron rich regions of the murine brain. This was paralleled with loss of short term memory, as evidenced by passive avoidance test, of the benzene treated animals.

Keywords: Benzene; Brain serotonin; Memory loss; Mice

1. Introduction

Vehicular emission has caused an abnormal increase in benzene in the environment, which is primarily absorbed in the body through inhalation. Apart from the hematotoxic (Aksoy, 1980; Yin et al., 1996; Rothman et al., 1996) and genotoxic effects (Tompa et al., 1994), chronic exposure to benzene has been related to certain neurological disorders manifested by altered functioning of the central and peripheral nervous system (Kyvik and Moen, 1995). The functioning of the nervous system is regulated by a delicate balance of the neurotransmitters among which serotonin play a pivotal role.

Central serotonin has been reported to control a variety of behaviors such as food intake, activity rhythm, sexual behavior and emotional states (Buhot et al., 2000). Oxidation product of serotonin, 5-5′ dihydroxy 4-4′ bitryptamine (DHBT) was suggested to have neurotoxic properties and contribute to neuronal damage (Huether et al., 1997). Neuronal cell death is the major cause of neurodegenerative disease. Alzheimer's disease is associated with degeneration of serotonergic neurons, with corresponding losses of 5-HT and transporter 5-HTT (Mossner et al., 2000; Sparks et al., 1988). It has been reported that history of exposure to organic solvents like benzene, toluene, phenols, alcohol, etc. has been shown to be associated with onset of the disease (Kukull et al., 1995).

Being lipophilic in nature, benzene and its metabolites concentrate in the nervous system causing imbalance in neurotransmitter levels (Hseih et al., 1990; Naskali et al., 1994). However, it is not known whether chronic exposure to benzene, is responsible for short term loss of memory and irritability in the exposed group. In the present study, the mice were chronically exposed to controlled doses of benzene to see whether the exposure caused any alteration in their memory performance and the status of central serotonin levels.

2. Materials and methods

2.1. Animals

Male Swiss mice 4–6 weeks of age were used for the study and were obtained from the Animal Care and Maintenance

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Department of the Institute. The animals were kept in plastic cages (six mice per cage) with alternate light and dark cycles of 12 h each. Food and water was given ad libitum.

2.2. Treatment

2.2.1. Benzene exposure

The mice were chronically exposed to benzene (Merk, India) by mixing it with drinking water, for a month. The animals were divided into two groups, one group receiving benzene 166 mg/l and the second group received 332 mg/l of benzene (Hseih et al., 1990). Animals receiving plain drinking water served as controls.

2.2.2. p-Chloroamphetamine

A group of animals were injected intraperitoneally with *p*-chloroamphetamine (Sigma Chemicals, USA), a serotonin synthesis blocker, at a dose of 10 mg/kg body weight (Farfel and Seiden, 1995). This group was given a single dose of injection 30 min prior to passive avoidance learning and served as positive control.

2.3. Dissection of brain tissue

After completion of the experimental schedule, animals were sacrificed and the brain areas were removed onto cold plates (4 °C). Tissue samples for each brain region were located and hypothalamus, raphe and caudate putamen were rapidly dissected, weighed and frozen immediately and stored at −80 °C untill assay.

Intact brain tissue were fixed in 10% formalin for histology and immuno-cytochemistry.

2.4. Determination of 5-HT concentration in brain of mice

Simultaneous detection of biogenic amines from brain tissue was done by HPLC with electrochemical detection. At the time of analysis different brain regions were homogenised with 50 μl of DHB and 100 μl of 0.1N HCl and centrifuged at 3000 rpm at 4 °C for 10 min. Twenty microliters of the supernatant was then injected into a reverse phase high performance liquid chromatographic coloumn (WATERS Novapak C18 column, 3.9 mm × 150 mm, coupled to an electrochemical detector WATERS 464 Pulsed ECD). The mobile phase consisted of 6.74 g citric acid, 4.81 g sodium citrate, 47 mg EDTA, 200 mg heptasulphonic acid, 1.15 ml glacial acetic acid, 3 ml tetrahydrofuran and 25 ml HPLC grade methanol. It was made to 1 l with HPLC grade water and pH adjusted to 4.9 using 10 M NaOH. The flow rate was set at 0.5 ml/min.

2.5. Passive avoidance test for memory

The procedure has been adopted from Chopin and Briley (1992). The test is based on the spontaneous tendency of rodents to avoid brightly illuminated areas. Emotional learning was tested in a step through avoidance task. The animals were placed in a chamber separated into two compartments. One compartment was brightly illuminated with a 60 W light bulb and the other was a dark compartment made of black wood and grided floor attached to the shock generator. The two compartments were separated by a sliding door made of wood. The room was dark during the experimental sessions, conducted between 9 a.m. and 3 p.m. Initially each animal was placed in the illuminated compartment and allowed to move freely for 30 s. The entrance to the dark compartment was then opened and as soon as the mice entered with all four paws on the grid floor, the door was closed and a single inescapable foot shock (0.8 mA/2 s) was delivered. After a 5 s delay the animals were returned to their home cages. Time spent by the animal to enter the dark compartment (=latency to enter the dark compartment) were recorded. The animals avoiding the dark compartment for 180 s were considered as remembering the passive avoidance task. The experiment was repeated after 48 h, 15 days, and 30 days of the initial training, in three groups of mice—those exposed chronically to benzene, positive controls which were injected with *p*-chloroamphetamine 30 min prior to learning and normal mice which served as negative controls.

Statistical analysis was done by Student's *t*-test.

3. Results

Neurotransmitters, especially serotonin (5-HT) play an important role in maintaining the homeostasis of the body. The mice were chronically exposed to 166 and 332 mg/l of benzene for a month. The experiment caused changes in the locomotor activity and restlessness in the exposed group of animals compared to controls. The concentration of serotonin was estimated in discrete regions of the brain on 15th day and after completion of the treatment.

Exposure to benzene (166 and 332 mg/l) for a month showed significant decrease in the concentration of serotonin in discrete regions of the brain especially raphe and hypothalamus, rich in serotonergic neurons and their innervations (Table 1). The decrease was more significant in the group receiving higher doses of benzene. With the increase in time period of exposure the decrease in 5-HT levels was more pronounced.

Entry latency was considered as the inborn escape behavior of rodents. The step-through latency of mice trained with electric foot shock was significantly higher than those trained without foot shock (Fig. 1). Normal mice exposed to a single session of foot shock did not step through the dark compartment for 180 s. This latency was nearly maintained for the entire experimental period. Interestingly, the animals chronically exposed to benzene showed a significant decrease in the step-through latency compared to the normal controls and the delay decreased with progression of time (Fig. 1). *p*-Chloroamphetamine treated mice also showed a remarkable