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Effect of Styrene Oxide on Levels of Biogenic Amines and Activity of Monoamine Oxidase in Different Parts of Rat Brain

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Styrene oxide, a metabolite of styrene is used as a diluent in epoxyresins and intermediate in the production of agricultural and biological chemicals, cosmetics, surface coatings and in the treatment of textile fibres (Lee and Neville 1967; IARC 1969). Exposure to styrene also results in the formation of styrene oxide through cytochrome P-450 mixed function oxidases in humans and other mammals (James and White 1967; Liebman and Oritz 1970; Ohtsuji and Ikeda 1971).

Industrial workers exposed to styrene suffer from peripheral and central nervous system (CNS) disorders (Seppalainen and Harkonen 1974). Styrene oxide has been reported to be a potent CNS depressant in experimental animals (Parkki et al. 1976). Styrene oxide being a reactive molecule is mainly considered to be responsible for the toxic effects of styrene on nervous system (Savolainen and Vainio 1977; Dixit et al. 1982).

Brain biogenic amines play a significant role in the functioning of the CNS. Therefore, levels of dopamine, noradrenaline and 5-hydroxytryptamine in discrete brain areas of rats exposed to styrene oxide were measured, to understand the mechanism of its CNS toxicity. Activity of monoamine oxidase, a major enzyme in the catabolism of these neurotransmitters was also estimated in the mitochondrial preparations of various brain regions.

MATERIALS AND METHODS

Male albino rats (150 $\pm$ 5g) of ITRC animal breeding colony, were given a commercial pellet diet (Hindustan Lever, Bombay) and water ad libitum. Styrene oxide (SO) in groundnut oil was administered intraperitoneally at doses equivalent to 25 mg and 50 mg/kg body weight and styrene at 50 mg/kg body weight

in the same manner for 14 consecutive days. The control animals received an equal volume of ground-nut oil in an identical manner. All the animals were killed 24 hours after the last treatment by cervical dislocation. Brains were removed, dissected into 7 regions on ice-cold glass plates according to the method of Iverson and Glowinski (1966).

The frozen tissue samples were weighed and homogenized in butanol acidified with 0.1 N HCl and centrifuged at 1500 rpm for 10 min. The clear supernatant was carefully recovered and used for estimation of noradrenaline, dopamine and 5-hydroxytryptamine (Jacobwitz and Richardson 1978). The activity of monoamine oxidase (MAO) was assayed in isolated mitochondria (Lovtrup and Zelander 1962) of brain regions (Tabor et al. 1955).

Protein content of the various brain regions was estimated by the method of Lowry et al. (1951), using bovine serum albumin as a reference standard.

RESULTS AND DISCUSSIONS

There was no mortality in control or treated animals during the period of the study. The treated animals also did not show any significant neurological deficits in comparison to controls.

The effect of styrene and SO on noradrenaline and 5-hydroxytryptamine contents in various parts of rat brain is shown in Table 1 and Table 2 respectively. Twenty four hours after the last injection of SO levels of noradrenaline (Table 1) were significantly increased in corpus striatum, cerebellum and cerebral cortex at both the doses. A similar dose dependent increase in levels of 5-hydroxytryptamine (Table 2) in corpus striatum, cerebral cortex and mid brain was also observed. Styrene at dose of 50 mg/kg caused no such change in the levels of these amines (Table 1 and 2). No effect of styrene and SO on levels of dopamine was observed at any of the dose levels used (data not shown).

Data presented in Table 3 shows that exposure to SO in rats significantly reduced MAO activity in mitochondrial preparations of hypothalamus at both the doses and in corpus striatum and cerebral cortex only at the higher dose. The observations that SO alters the level of brain noradrenaline

Table 1 Effect of styrene oxide and styrene on regional levels of noradrenaline (/ug/g fresh tissue)

Treatment	Cerebellum	Pons medulla	Mid brain	Hypothalamus	Hippocampus	Corpus striatum	Cerebral cortex
Control	0.19±0.004	0.35±0.026	0.38±0.012	1.66±0.07	0.25±0.003	0.22±0.009	0.12±0.007
Styrene Oxide (25 mg)	0.24±0.004*	0.36±0.026	0.41±0.020	1.79±0.028	0.25±0.009	0.26±0.002*	0.18±0.01*
Styrene Oxide (50 mg)	0.25±0.002*	0.37±0.017	0.42±0.022	1.77±0.032	0.25±0.006	0.27±0.002*	0.18±0.01*
Styrene (50 mg)	0.19±0.003	0.36±0.010	0.39±0.014	1.60±.036	0.25±0.003	0.22±0.004	0.13±0.008

All values are expressed as mean ± S.E. from six animals.
*P /0.05; (Student's 't' test).

Table 2 Effect of styrene oxide and styrene on regional levels of 5-hydroxytryptamine (/ug/g fresh tissue)

Treatment	Cerebellum	Pons medulla	Mid brain	Hypothalamus	Hippocampus	Corpus striatum	Cerebral cortex
Control	0.33±0.007	1.03±0.032	0.87±0.053	1.86±0.09	0.52±0.028	0.99±0.044	0.40±0.014
Styrene Oxide (25 mg)	0.34±0.006	0.95±0.035	1.06±0.046*	2.12±0.045*	0.47±0.029	1.30±0.044*	0.44±0.005*
Styrene Oxide (50 mg)	0.35±0.005	1.15±0.117	1.15±.078*	2.02±0.053	0.54±0.034	1.65±0.138*	0.53±0.010*
Styrene (50 mg)	0.34±0.006	0.95±0.03	0.86±0.048	1.90±0.088	0.55±0.022	0.98±0.047	0.42±0.008

All values are expressed as mean ± S.E. from six animals.
*P /0.05 (Student's 't' test).

Table 3 Regional monoamine oxidase* activity of control, styrene oxide and styrene treated rats

Treatment	Cerebellum	Pons medulla	Mid brain	Hypothalamus	Hippocampus	Corpus striatum	Cerebral cortex
Control	1.64±0.088	1.70±0.16	2.67±0.075	3.98±0.03	3.68±0.20	1.73±0.03	1.74±0.115
Styrene Oxide (25 mg)	1.59±0.10	1.62±0.09	2.30±0.119	3.60±0.27	3.25±0.20	1.50±0.114	1.33±0.02**
Styrene Oxide (50 mg)	1.58±0.10	1.56±0.009	2.19±0.21	2.31±0.108**	1.92±0.16**	1.25±0.09**	1.17±0.105**
Styrene (50 mg)	1.53±0.04	1.59±0.13	2.71±0.19	3.10±0.23	2.79±0.28	1.68±0.13	1.68±0.09

Data represents mean ± S.E. of 6 animals.
*n moles of benzaldehyde formed/min/mg protein.
**P $\leq$ 0.05 (Student's 't' test).

and 5-hydroxytryptamine without affecting the dopamine levels suggests that styrene oxide may not be acting by inhibiting MAO in CNS neurons. These observations are consistent with our earlier studies with styrene (Husain et al. 1980). Similar increases in levels of biogenic amines associated with decrease in MAO activity have also been observed with certain other neurotoxic chemicals (Dixit et al. 1981; Singhal and Merali 1979).

The SO induced alterations, in noradrenaline and 5-hydroxytryptamine levels, in certain localised areas of brain, may be due to their increased turnover rates or impairment of their active transport. Noradrenaline and 5-hydroxytryptamine play an important role in a number of functions such as onset of sleep, mood, arousal, temperature regulation and sensory perception, etc. Changes in their levels have been shown to result in abnormal mental behaviour and mood (Singhal and Merali 1979; Curzon 1972). Corpus striatum and cerebellum appear to be sensitive to SO as levels of both the amines were altered in these areas. Corpus striatum is involved in cerebral lateralization and cerebral cortex in exploratory and locomotor activity to certain extent. Hence alterations in the level of noradrenaline and 5-hydroxytryptamine observed in this study suggest their role in the CNS disorders reported in styrene exposed workers (Seppalainen and Harkonen 1974). Many xenobiotics such as aromatic hydrocarbons, acrylamide and manganese are reported to induce CNS disorders by affecting the neurotransmitter function of biogenic amines (Dixit et al. 1981; Curzon 1972; Anderson et al. 1981; Chandra et al. 1979).

In our earlier studies we have observed that administration of styrene at a dose of 1000 mg/kg body weight orally for 14 consecutive days caused an increase in the levels of 5-hydroxytryptamine and noradrenaline and decreased the MAO activity in whole brain. In the present study, no such effect of styrene was evident on level of 5-hydroxytryptamine, noradrenaline and MAO activity in any brain region at 25 and 50 mg/kg dose. These results indicate that in comparison to styrene its metabolite SO plays a major role in its neurotoxicity. Perhaps, the levels of SO formed after administration of such low doses of styrene are not sufficient to produce alterations in the levels of these biogenic amines.

In conclusion, these results suggest that alterations in the levels of noradrenaline and 5-hydroxytryptamine may be responsible for neurotoxicity of SO and provide further support to the view that neurotoxicity of styrene may be mediated through its epoxide styrene oxide.

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