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Effect of Styrene Oxide on Dopamine Receptors in Rats

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Styrene oxide (SO) has several industrial applications. It is extensively used as a reactive diluent in epoxy resins to reduce the viscosity of mixed system prior to curing. It is also used as an intermediate in the preparation of variety of agricultural and biological chemicals, cosmetics, surface coatings, treatment of textiles and fibres and as a raw material for the production of phenyl stearyl alcohol in perfume industries (IARC, 1979).

Due to wide spread use of SO, industrial workers and general population can be exposed to this chemical directly. Exposure to styrene also leads to the formation of SO in humans and other mammals (Leibman and Ortiz 1970). It has been reported that SO is mostly responsible for the toxic action of styrene (Savolainen and Vainio 1977).

Involvement of central nervous system in styrene toxicity has been suggested in humans and laboratory animals (Lorimer et al. 1976; Parkki et al. 1976). Our previous studies have shown a significant increase in the dopamine receptors in the striatal membranes of rats treated with styrene (Agrawal et al. 1982). In order to study whether the active metabolite, styrene oxide, also alters the sensitivity of dopamine receptors, its effect was studied on dopamine receptor binding in rats after single and repeated exposure.

MATERIALS AND METHODS

Male albino rats of Industrial Toxicology Research Centre, Lucknow, Breeding colony, maintained on commercial pellet diet (Hindustan Lever, Ltd., India) under standard laboratory conditions were used in the present study. In single exposure study, eighteen rats were equally divided in three groups and treated intraperitoneally with 0, 50 and 100 mg/kg SO(dissolved in groundnut oil). In repeated exposure study 36 rats were divided equally in three

groups and 0, 25 and 50 mg/kg of SO was administered daily for 14 consecutive days. Six animals from each group were sacrificed 24 hours after the last dose. The remaining 6 animals in each group in the repeated exposure studies were left untreated after the last dose and sacrificed after 14 days,to study the reversibility of the effect.

The brains were removed and corpus striata were dissected out immediately by the method of Glowinski and Iversen (1966) and frozen at -20°C till membrane preparation. Crude synaptic membranes were prepared by homogenizing the tissue in 0.32 M sucrose followed by centrifugation at 50,000 g for 10 minutes. The pellet was resuspended in cold water and centrifuged at the same speed. The final pellet was suspended in 40 mM Tris HCl buffer pH 7.4 at a concentration corresponding to 50 mg original wet weight of tissue/ml (Creese and Snyder, 1978).

Binding of ^{3}H-spiroperidol (a specific ligand for labeling of dopamine receptor) to corpus striatal membrane was assayed as described earlier (Seth et al. 1981). In brief, binding was carried out by incubating the crude synaptic membranes (300-400 /ug protein, Lowry et al. 1951) together with 10^{-9}M spiroperidol (22 Ci/mmol NEN) in presence of 40 mM Tris HCl buffer pH 7.4 at 37°C for 15 minutes. Parallel assays were carried out in the presence of 10^{-6}M haloperidol to determine the extent of nonspecific binding. The receptor- ligand complex was separated by filtering the incubation mixture on glass fibre discs (pore size 0.3 /u, Gelman Inc. Ann Arbor MI USA). The filter discs were washed rapidly three times with 5 ml of cold tris buffer and counted in scintillation mixture (PPO + POPOP + Toluene + Dioxan + Methanol + Naphthalene) in LKB Wallac Rack beta II scintillation counter at an efficiency of 50% for tritium. The specific binding was calculated by subtracting nonspecific binding from total binding obtained in absence of haloperidol. Results are expressed in terms of pmoles ^{3}H-spiroperidol bound per gram protein. The method used, is essentially similar to other filtration binding methods (Bennett1978). Basic binding characteristics like delineation of saturability, regional distribution, specificity and reversibility were established prior to this study (Agrawal et al. 1981).

Differences between control and experimental groups were tested using Fisher least significant difference test. The accepted level of significance in all cases was $P < 0.05$, using two tailed test.

RESULTS AND DISCUSSION

Specific binding of ^{3}H-spiroperidol to the corpus striatal

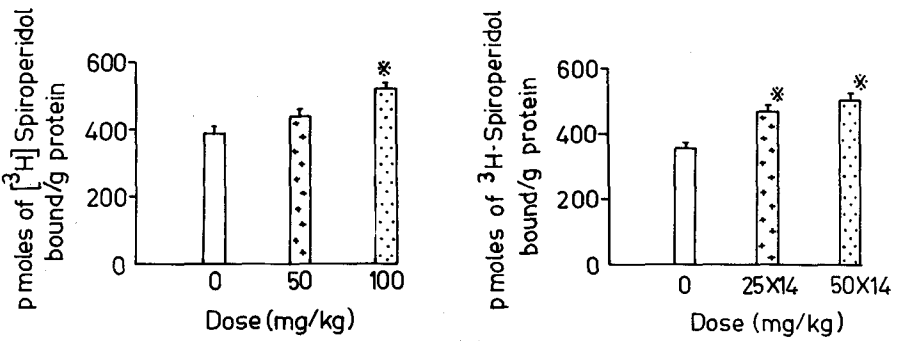

Figure 1a Effect of single exposure of styrene oxide on ^{3}H-spiroperidol binding to corupus striatal membrane. Data are mean ± S.E. from six animals (Left).

1b Effect of repeated exposure of styrene oxide on ^{3}H-spiroperidol binding of rat corpus striatal membrane (Right). Data are mean ± S.E. from six animals.
*Differs significantly from controls. ($P < 0.05$ Fisher's Least Significant Difference test).

membranes of control and styrene oxide (SO) treated animals is shown in Figure 1a and 1b.

Rats exposed to single higher dose of SO (100 mg/kg), exhibited a significant increase in binding of ^{3}H-spiroperidol to striatal membranes in comparison to controls, while the animals receiving the lower dose (50 mg/kg) of SO showed no such effect. The repeated exposure to styrene oxide (25 and 50 mg/kg) for 14 consecutive days caused a significant increase in the binding of ^{3}H-spiroperidol at both the dose levels. These results indicate a significant increase in dopamine receptor activity following SO treatment. Such an increase in receptor binding could be due to alteration in the number of receptor sites or the affinity of the receptor.

It is evident from the results of Scatchard plots (Fig. 2) (Scatchard 1949) that SO exposure caused a significant effect on the maximum number of binding sites (Bmax control =573 ± 28.7, Bmax treated=810 ± 43.2 pmoles bound/g protein) without any significant effect on the affinity of the receptor (K_D control=0.58 ± 0.02 nM and K_D treated=0.66 ± 0.042 nM). The effect of SO on dopamine receptor appears to be reversible as no significant change in ^{3}H-spiroperidol binding was detectable between control and treated groups

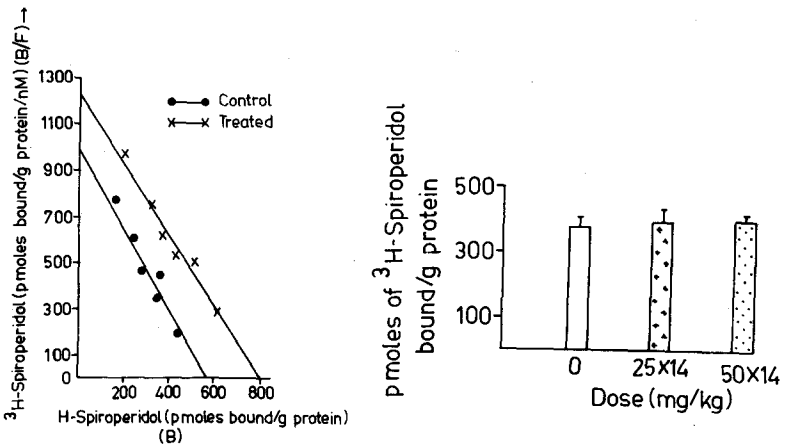

Figure 2. Scatchard analysis of binding of ^{3}H-spiroperidol to corpus striatal membranes after repeated exposure of styrene oxide. Each data point of saturation curve is mean of three separate experiments carried out in triplicate (Bmax control=573 ± 28.7; Bmax treated= 810 ± 43.2 pmoles bound/g protein. K_D control =0.58 ± 0.02 nM, K_D treated=0.66 ± 0.042 nM).

Figure 3. ^{3}H-spiroperidol binding to corpus striatal membrane prepared from rats 14 days after last SO dose. Experimental details are given in the text. Data are mean ± S.E. from six animals.

14 days after cessation of the treatment (Figure 3).

Pharmacological agents or surgical procedures are known to alter the density of striatal dopamine receptors without affecting the affinity of the receptor (Creese et al. 1977; Rosengarten and Friedhoff 1979). Such a change in the density of binding sites appears to be the characteristic response leading to denervation supersensitivity (Waddington and Cross 1978). The increase in total number of receptor sites (Bmax) without any significant change in the affinity of the receptor (K_D) following SO treatment suggests denervation type of supersensitivity of dopamine receptor in the present study.

Since no significant effect of styrene oxide was observed on striatal dopamine content (Husain et al. unpublished work), the supersensitivity of dopamine receptor observed in the present study may be due to alterations in the rate of uptake or release of the amine through the activation of presynaptic receptors (Bymaster and Wong 1977). The observed supersensitivity could also be due to the destruction of dopamine neurons of nigrostriatal pathway by SO exposure, as has been observed with other pharmacological agents (Ungersted 1971).

These results suggest that styrene oxide, like styrene, may also be exerting its neurotoxicity through dopaminergic transmission.

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