Pharmacology Biochemistry and Behavior, Vol. 66, No. 3, pp. 523–532, 2000
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0091-3057/00/$–see front matter

PII S0091-3057(00)00216-1

Altered Regulation of Dopaminergic Activity and Impairment in Motor Function in Rats After Subchronic Exposure to Styrene

SAROJ K. CHAKRABARTI

Département de médecine du travail et d'hygiène du milieu, Faculty of Medicine, Université de Montréal, P.O. Box 6128, Main Station, Montréal, Québec H3C 3J7, Canada

Received 3 November 1998; Revised 3 December 1999; Accepted 16 December 1999

CHAKRABARTI, S. K. *Altered regulation of dopaminergic activity and impairment in motor function in rats after subchronic exposure to styrene.* PHARMACOL BIOCHEM BEHAV **66**(3) 523–532, 2000.—Animal and human studies suggest a dopamine-mediated effect of styrene neurotoxicity. However, the results reported to date are incomplete and not consistent. As such, the mechanism of its neurotoxicity is still unclear. The present study has, therefore, reexamined the central dopaminergic system in relation to some neurobehavioral effects in rats following subchronic exposure to styrene. Groups of adult male Sprague–Dawley rats received 0, 0.25, or 0.5 g styrene per kg b.wt. by gavage for 13 consecutive weeks. Twenty-four hours after cessation of such treatment with the higher dose (0.5 g/kg), the contents of dopamine (DA) and its metabolites were significantly reduced in the corpus striatum, hypothalamus, and lateral olfactory tract regions. In vitro styrene showed a significant increase in DA release from rat striatal synaptosomes similar to that of tyramine. Significant loss of motor function was observed on days 56, 70, and 84 during the styrene treatment with the higher dose, and lasted over a month after such treatment. However, the treated animals recovered their motor function within 45–60 days after cessation of such treatment, along with the recovery of normal levels of dopamine and its metabolites. Furthermore, styrene-induced initial impairments in measures of dopaminergic activity cannot be attributed to altered regulation of tyrosine hydroxylase activity. Specific [^{3}H]-spiroperidol binding was also unaltered 7 or 15 days after subchronic treatment with styrene. These data imply that despite the dopaminergic neuronal loss due to styrene, dopaminergic transmission was not reduced to a level that would result in an overall development of dopamine receptor supersensitivity in the striatum. Collectively, these studies indicate that the subchronic neurotoxic action of styrene may be primarily presynaptic in nature and may involve impaired regulation of DA content and stimulation of DA release. © 2000 Elsevier Science Inc.

Styrene Subchronic neurotoxicity Dopaminergic system Motor function Uptake and release Receptor binding Rats

SYMPTOMS of neurological disorders involving both the central and peripheral nervous system have been reported in workers exposed to styrene monomer during manufacturing-reinforced plastic products containing polystyrene (3,18). Styrene is also reported to leach out from the finished plastic materials. As such, migration of the styrene monomer into food material stored in polystyrene plastic containers has caused great concern due to their toxic properties (33). The levels of residual styrene monomer in the range of 70–2260 ppm have been detected in polystyrene food ontainers (31). Thus, people might be potentially exposed to styrene ranging from low to very high toxic concentrations via the oral route.

The pharmacokinetics of styrene in humans is similar to that reported in laboratory animals (4). Ninety percent of the dose was excreted in the urine in 24 h. Only 2% of the dose was found in feces (34) following an oral dose of 20 mg ^{14}C-styrene/kg. When Wistar rats were given IV 4 or 13 mg styrene/kg b.wt., a rapid distribution of styrene to major organs including heart, lung, spleen, kidney, and brain was observed. Furthermore, relatively high levels of styrene were found in the rat brain compared to other tissues examined, suggesting this tissue might be a target tissue for styrene toxicity (35). The half-lives for elimination of styrene from blood were found to be 2.7 min (first phase) and 7.7 min (late phase). But when styrene monomer in aqueous solution was administered to adult male Wistar rats by gastric intubation, the elimination half-lives of styrene from blood were found to be 16.9 min (fast phase) and 47.1 min (slow phase) (33).

The biotransformation of styrene in humans is qualitatively similar to that in laboratory animals (4). The biotrans-

formation of styrene involves initial formation of reactive styrene-7,8-epoxide by microsomal mixed-function oxidase system containing cytochrome P-450. This reactive epoxide is then detoxified either to styrene glycol by microsomal epoxide hydratase, or to glutathione S-conjugates by soluble glutathione S-transferases. This glutathione S-conjugates are finally degraded to mercapturic acids before being excreted to urine (17).

A wide variety of barely detectable to severe adverse acute neurologic effects of occupational styrene exposure have been reported involving decreased nerve conduction velocities and electroencephalographic, functional, and psychiatric impairments (6,10,12,36). Toxicity of the CNS due to occupational exposures to styrene in excess of 100 ppm in air has been well established, although the effects at lower concentrations of styrene in the workplace are also possible (30). Despite the study of workers exposed for many years, no indications of persisting damage to the nervous system were evident (24).

However, the exact mechanism of styrene-induced neurotoxicity is still unknown. Although animal and human studies suggest a dopamine-mediated effect of styrene-induced neurotoxicity, the results reported to date have not been consistent. Thus, in one study, dose-dependent depletions in striatal and tubero-infundibular dopamine (DA) concomitant with increases in homovanillic acid contents in the same areas due to short-term very high exposure to styrene have been reported; such data are found to be inconsistent with the observed inhibition of tyrosine hydroxylase (20). On the other hand, increased levels of serotonin (5-HT) and norepinephrine (NE) as well as reduced activity of monoamine oxidase have been observed in the whole brain of rats treated orally with styrene, but without any effect on DA levels (15). Dopamine depletion has been observed in the brains of styrene-treated rabbits (21,26) and an increase in DA receptor binding in rat brain striatum, possibly as a compensatory reaction to DA depletion, has also been reported (37). Dose-related increases in serum prolactin and thyroid hormone concentrations among workers exposed to styrene have been attributed to a toxic mechanism involving reduction of tuber-infundibular DA content (2,20). Color vision loss (an early appearance) due to styrene (13) has been suggested to be due to an interference of styrene on dopaminergic mechanism of retinal cells, as suggested for other neurotoxic effects (22). The present study has, therefore, reexamined the effects of subchronic exposure to styrene on the central dopaminergic system and its relationship to some neurobehavioral effects using rat as an animal model.

METHOD

Adult male Sprague–Dawley rats (Charles River Canada inc., St. Constant, Quebec) weighing 160–180 g were used. They were kept in a room maintained at constant temperature (22°C) with a 12 L:12D cycle and were fed Purina Laboratory chow and tap water ad lib. Gamma-butyrolactone (GBL), NSD1015, bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO). TL-99 was purchased from Research Biochemicals, Inc. (Natick, MA). Styrene (99%) was obtained from Sigma-Aldrich Canada, Limited (Mississauga, Ontario). All other reagents are of highest commercial grade, unless otherwise mentioned.

In this study, the doses of 0.25 and 0.5 g styrene per kg b.wt. for subchronic oral administration were chosen based on the following information. Repeated oral administration of styrene at 200 and 400 mg/kg for 90 days produced a significant increase in the specific binding of ^{3}H-spiroperidol to rat striatal membranes 24 h following the last dose (1). However, no significant effect was seen on body weights and striatal weights due to such styrene exposure (1). Adult male Albino rats when treated with 200 or 400 mg styrene per kg b.wt. by gavage 6 days a week for 100 days, showed significant dose-dependent hepatotoxic effects including an increase in the activity of some liver microsomal drug-metabolizing enzymes as well as a decrease in glutathione-S-transferase, mitochondrial succinic dehydrogenese and β-glucuronidase activities (29). Besides, very high levels of styrene monomer up to 2260 ppm have been found in polystyrene food containers (31). Based on this information, two subchronic oral doses of styrene, for example, 0.25 and 0.5 g per kg b.wt. have been chosen for the present study. These doses of styrene are considerably higher than human exposure of 100 ppm.

In a first series of the experiment, each group of control and treated rats consisted of 20 rats. Groups of rats received 0.25 and 0.5 g per kg b.wt. per day of styrene in corn oil by gavage, 7 days a week, for 13 consecutive weeks. Control group received an equal volume of corn oil (1 ml/kg). During the treatment period, food and water intake as well as body weights were measured. Furthermore, changes in physical conditions, if any, were also noted. One-half of the animals in each group (i.e., 10 animals per group) was used for neurobehavioral studies at different days during 13 weeks of styrene treatment as well as at different days following the end of such styrene treatment. Twenty-four hours after the end of such treatment with styrene, the remaining half of the animals in each group was sacrificed for removal of brain tissue. The brain tissue was immediately collected on ice, and rapidly frozen in liquid nitrogen (−80°C) and the following brain regions were dissected: cortex, cerebellum, corpus striatum, hypothalamus, and lateral olfactory tract, following the method of Glowinski and Iversen (11). The synaptosomes of these brain regions were then prepared using the method of Gray and Whittaker (14) as modified by Dodd et al. (9), and were conserved at −80°C under liquid nitrogen until analyzed for biogenic amines and their principal metabolites. At the end of the behavior experiments (i.e., 60 days after the end of 13-week subchronic treatment with 0.5 g styrene/kg), six animals from each group of control and styrene-treated rats were sacrificed for removal of brain tissues. The analyses of DA, and its principal metabolites DOPAC and HVA, were then carried out following the same protocol as mentioned above.

Analyses of Brain Biogenic Amines and Their Metabolites

The biogenic amines: DA, NE, 5-HT, and their metabolites, for example, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid, (5-H1AA), and vanillylmandelic acid (VMA) were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD) (27). The mobile phase contained 100 mM NaH_2PO_4, 2.6 mM octyl sodium sulfate (Kodak, Rochester, NY), 0.1 mM sodium ethylene diaminetetraacetic acid (Sigma, St.Louis, MO), 0.25 mM triethylamine, and 16% methanol as an organic modifier. The pH of the mobile phase was adjusted to pH 4.25 with 3 M H_3PO_4, and changed in small increments to maintain baseline resolution of the compounds of interest. The mobile phase was delivered at a flow rate of 1.0 ml/min by a Waters 6000A chromatography pump (Waters, Milford, MA) to a single C-18 5-μm reverse-phase column (Supelcosil LC-18-DB, Supelco, PA) maintained at 35°C. The sensitivity of the assays of various

amines and their metabolites was 25–30 ng/ml, and the assay coefficient of variation was in the range of 3.2–5.3%.

Tyrosine Hydroxylase Activity

A primary manifestation of regulation of DA synthesis at nerve terminals is the inhibition exerted on tyrosine hydroxylase (TH) activity via dopaminergic autoreceptors. To this end, a pharmacological model has been used extensively to assess the functional status of these autoreceptors based on prior administration of a DA agonist to dose dependently inhibit the gamma-butyrolactone (GBL)-induced increase in TH activity (32). So, in another set of experiments, this study was conducted to assess the functional integrity of receptor-mediated regulation of DA synthesis in corpus striatal and hypothalamic synapses of subchronically styrene-treated (0.5 g/kg) (six rats/group) rats for 13 weeks by measuring TH activity in the GBL-DA agonist paradigm. The DA autoreceptor agonist, 6,7-dihydroxy-2-dimethyl-aminotetralin (TL-99) has been shown to be effective in this pharmacological paradigm and relatively selective in its action for presynaptic, as opposed to postsynaptic DA receptors (7,19). Rats were given saline or the DA autoreceptor agonist TL-99 in doses of 2.5, 5, and 10 mg/kg, IP 15 min before GBL (750 mg/kg, IP) followed 15 min later by the amino acid decarboxylase inhibitor NSD1015 (100 mg/kg, IP) 30 min before final treatment. Animals were then sacrificed 30 min later after the final treatment. Drugs were dissolved in deoxygenated isotonic saline containing 0.1% sodium metabisulfite and administered in a volume of 1 ml/kg. This drug paradigm causes a linear accumulation across time of the product of TH activity, L-DOPA, by blocking the decarboxylation of L-DOPA to DA. Tissues were homogenized and samples prepared as described above for determination of DA and its metabolites except that alpha-methyl-DOPA was used as an internal standard. Chromatographic parameters were also identical except for mobile phase pH was set to 2.5 to cause elution of L-DOPA away from the void volume. For all assays, protein content was determined by the method of Bradford (5), using bovine serum albumin as a standard. DA uptake and release in rat striatal synaptosomes in vitro.

In a separate experiment, DA uptake and release assays were carried out in the rat striatal synaptosome preparation and in the presence of 100 μM pargyline to prevent the chemical degradation of DA. Mazindol is a well-characterized strong inhibitor of DA uptake by virtue of its binding associated with neuronal DA uptake sites in corpus striatum membranes (16). Therefore, the inhibition of DA uptake by styrene/styrene oxide (a metabolite of styrene) was measured in the rat striatal synaptosomes and, hence, compared with that of mazindol, as expressed as percent of mazindol inhibition.

To determine DA uptake, striata from 10–15 rats were isolated and homogenized in 0.9 ml of Tris HCl (50 mM)-sucrose (0.3 M) buffer, pH 7.5. The homogenate was centrifuged at $1000 \times g$ for 20 min at 4°C. The resultant supernatant was further centrifuged at $27000 \times g$. The pellet was resuspended in 0.9 ml of the same buffer and used as the crude striatal synaptosome preparation. The uptake assay medium contained Tris HCl (50 mM), pH 7.5, sucrose (0.3 M), KCl 5 mM, NaCl 120 mM (assay buffer), pargyline 100 μM (MAO inhibitor), mazindol/styrene/styrene oxide each 100 μM, and striatal synaptosomes (1 mg protein). DA (50 μM) was then added to the assay tube, and was incubated at 37°C for 5 min. The dose of 100 μM was determined based on a maximum inhibition of DA uptake in rat striatal synaptosomes by 100 μM mazindol (unpublished results). The reaction was terminated by adding 3 ml of ice-cold assay buffer to the sample, and the content was immediately (within 3 s) filtered through a buffer saturated GF/B filter (Whatman Inc.) under vacuum. The filter was washed with additional 6 ml of buffer. DA was extracted from the filter and the aliquot of the extract was injected into the liquid chromatograph and DA was quantitated by an HPLC method described above. The inhibition of specific DA uptake by styrene/styrene oxide was expressed as percent of mazindol inhibition.

The DA release assay was carried out in two steps. The first step involved DA uptake in striatal synaptosomes as described above. The GF/B filter containing synaptosomal tissues preloaded with DA was transferred to a tube containing the assay buffer, pargyline (100 μM), and the test compounds tyramine, styrene, and styrene oxide, each 100 μM in a total volume of 5 ml. The filter was incubated at 37°C for 5 min. The medium was filtered again over the same filter, which was washed three times with 3 ml of assay buffer each time. Finally, the filter paper was extracted with solvent and DA was quantified as before. The data were expressed as the mean of DA remaining in the synaptosomal tissue (pmol/mg/protein/5 min incubation ± SE) and also as the percentage of DA release with reference to the effect of tyramine, a DA releasing agent.

In another separate experiment, receptor binding study was carried out 7 and 30 days after termination of in vivo subchronic (13-week) treatment with styrene. Binding of ^{3}H-spiroperidol (a specific ligand for labeling dopamine receptor) to a corpus striatal membrane was estimated using the method described by Seth et al. (28). Briefly, binding was carried out by incubating the crude synaptic membranes containing 500 μg protein plus 10^{-9} M spiroperidol (0.03–0.8 nM; ^{19}Ci/mmol, Amersham) in the presence of 40 mM Tris HCl buffer pH 7.4 at 37°C for 15 min. Parallel experiments were carried out in the presence of 10^{-6} M haloperidol to determine the amount of nonspecific binding. The receptor-ligand binding complex was separated by rapid vacuum filtration. The filter discs were then washed rapidly three times with cold Tris-HCl buffer, and finally counted on a scintillation counter. The specific binding was calculated by subtracting nonspecific binding from total binding obtained in the absence of haloperidol. Results are expressed in terms of pmol ^{3}H-spiroperidol bound per gram of protein. Data were analyzed using Scatchard plots to give B_{max} and K_d values. Regression coefficient ranged between 0.97 and 0.99.

Neurobehavioral Study

Because animal and human studies suggest a dopamine-mediated effect of styrene neurotoxicity, behavioral tests involving motor function have been chosen in this study.

Tests of Motor Function

Motor activity was measured for 1 h in 16 figure—eight mazes as previously described (25), each consisting of a series of interconnected alleys (10×10 cm) converging on a central arena and covered with transparent acrylic plastic. Eight phototransistor/photodiode pairs were used to detect motor activity. Each time a photobeam was interrupted, one activity count was recorded. Control and two styrene-treated groups each containing 10 rats were tested. Animals were tested individually for 1 h and were transported to the maze room 5 min prior to testing individually.

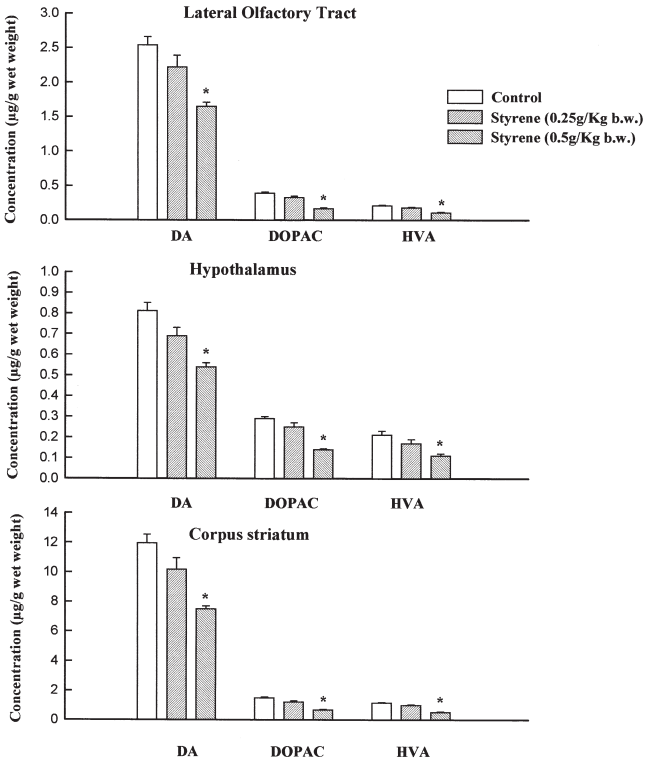

FIG. 1. Effects of subchronic treatment (13 weeks) of rats with styrene on the contents of DA, and its principal metabolites, DOPAC and HVA in the corpus striatum, hypothalamus, and lateral olfactory tract regions measured 24 hr after the end of such treatment. Results are averages ± SE of 10 rats per group. *Indicates significantly different from control, $p < 0.05$. Error bars not seen in some figures are hidden with the average values.

Gait Analysis

The hind paws of each rat were pressed on a pad containing black ink, then placed in the closed end of a 10 × 10 × 95 cm alley, the floor of which was covered with white paper. The rat was then allowed to walk through the alley until to the open end. Trials were repeated until six steps were registered with a new blotter used as required. Two measurements were made: (a) stride length—the distance in millimeters between consecutive ipsilateral footprints with reference to the notch between the pads at the base of the second and third digits of the hind paws (two right and two left strides were averaged); (b) stride width—the perpendicular distance in millimeters from a hindlimb footprint on one side to the line between successive hindlimb steps on the other side (four widths were averaged) (23).

Landing Hindlimb Foot Splay

Animals were tested for landing foot-spread as a measure of motor function (motor coordination) following the procedure of Pryor and Rebert (23). The hindpaws were inked as mentioned above. Each animal was held under the shoulders and dropped from a height of 30 cm onto a surface covered with white paper. The distance in millimeters between the hind feet was measured from the base of the second and third digits of each foot (four trials were averaged).

Statistics

Data were expressed as means ± SEM. The data were subjected to a repeated-measures one-way analysis of variance to test for significant changes within each individual group over time. Difference between groups of data obtained at the same time point were compared by ANOVA. Neurochemical parameters were analyzed by ANOVA followed by Duncan's test for multiple comparisons. ANOVA with repeated-measures analysis followed by Tukey's multiple comparison test for post hoc analysis was carried out with the data obtained for motor activity tests. *p*-Values less than 0.05 were taken as the level of significant difference.

RESULTS

Effects of Styrene Exposure on Body Weights

Exposure of rats to 0.25 and 0.5 g styrene per kg body weight for 13 weeks did not show any significant change in the body weights in comparison to the control animals. The treated animals weighed, on average, 5–10% less than control but these differences were not significant.

TABLE 1

LEVELS (μg/g) OF 5-HT, 5-HIAA, NE, AND VMA IN VARIOUS BRAIN REGIONS FOLLOWING EXPOSURE OF RATS TO STYRNE FOR 13 WEEKS

Region	Group	5-HT	5-HIAA	NE	VMA
Corpus striatum	Control	0.52 ± 0.05*	0.41 ± 0.03	0.31 ± 0.03	0.18 ± 0.01
	0.25g/kg	0.56 ± 0.07	0.36 ± 0.04	0.29 ± 0.03	0.21 ± 0.02
	0.50g/kg	0.51 ± 0.44	0.33 ± 0.04	0.27 ± 0.02	0.23 ± 0.02
Hypothalamus	Control	1.14 ± 0.10	0.63 ± 0.06	1.10 ± 0.1	0.43 ± 0.05
	0.25g/kg	0.98 ± 0.10	0.68 ± 0.07	1.4 ± 0.1	0.46 ± 0.05
	0.50g/kg	0.91 ± 0.10	0.70 ± 0.07	1.6 ± 0.2	0.41 ± 0.04
Lateral olfactory tract	Control	0.21 ± 0.02	0.14 ± 0.01	0.46 ± 0.02	0.10 ± 0.005
	0.25g/kg	0.26 ± 0.02	0.16 ± 0.01	0.36 ± 0.02	0.08 ± 0.004
	0.50g/kg	0.18 ± 0.01	0.11 ± 0.02	0.50 ± 0.02	0.13 ± 0.01

5-HT = 5-hydroxytryptamine, 5-H1AA = 5-hydroxyindoleacetic acid, NE = norepinephrine, VMA = vanillylmandelic acid.

*Results are averages ± SE of 10 animals.

TABLE 2

LEVELS (μg/g) OF VARIOUS BIOGENIC AMINES AND THEIR METABOLITES IN CEREBRAL CORTEX OF RATS FOLLOWING EXPOSURE TO STYRENE FOR 13 WEEKS

Biogenic Amines and Metabolites	Control	0.25 g Styrene per kg b.wt.	0.50 g Styrene per kg b.wt.
DA	0.37 ± 0.02*	0.33 ± 0.02	0.30 ± 0.03
DOPAC	0.14 ± 0.01	0.12 ± 0.01	0.11 ± 0.02
HVA	0.06 ± 0.005	0.05 ± 0.004	0.07 ± 0.005
5-HT	0.61 ± 0.07	0.56 ± 0.06	0.47 ± 0.04
5-H1AA	0.23 ± 0.03	0.24 ± 0.02	0.27 ± 0.03
NE	0.29 ± 0.02	0.26 ± 0.02	0.34 ± 0.03
VMA	0.23 ± 0.02	0.20 ± 0.01	0.18 ± 0.02

*Results are averages ± SE of 10 animals.

TABLE 3

LEVELS (μg/g) OF VARIOUS BIOGENIC AMINES AND THEIR METABOLITES IN RAT CEREBELLUM FOLLOWING EXPOSURE TO STYRENE FOR 13 WEEKS

Biogenic Amines and Metabolites	Control	0.25 g Styrene per kg b.wt.	0.50 g Styrene per kg b.wt.
5-HT	0.100 ± 0.01*	0.12 ± 0.01	0.15 ± 0.02†
5-H1AA	0.08 ± 0.01	0.06 ± 0.01	0.09 ± 0.01
NE	0.145 ± 0.01	0.161 ± 0.01	0.11 ± 0.01
VMA	0.08 ± 0.01	0.05 ± 0.01	0.06 ± 0.01

*Results are averages ± SE of 10 animals.
†Significantly different from control, $p < 0.05$.

Effects on Neurotransmitter Levels and Their Metabolites

Subchronic exposure of rats to styrene at a dose of 0.5 g/kg for 13 weeks led to a significant decrease in dopamine (DA) concentrations in the corpus striatum, hypothalamus, and lateral olfactory tract regions, while no such effects could be seen when exposed to styrene at a dose of 0.25 g/kg (Fig. 1). The major metabolites of DA, for example, 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) also were decreased significantly following similar exposure to 0.5 g styrene per kg b.wt., whereas no such changes were observed in rats exposed to 0.25 g styrene per kg (Fig. 1). Neuronal turnover, estimated by determining the ratios of the metabolites to neurotransmitters, was also reduced by exposure to styrene, in a dose-dependent manner. No statistically significant changes in the levels of 5-hydroxytryptamine (5-HT) and norepinephrine (NE) or their metabolites were observed in the corpus striatum, hypothalamus, and lateral olfactory tract regions (Table 1). Except a significant increase in 5-HT in cerebellum at the higher dose, neither significant changes in the levels of catecholamines and their metabolites, nor those of 5-HT and 5-H1AA were seen in the cerebral cortex and cerebellum following such subchronic exposure to styrene (Tables 2 and 3). The contents of DA, DOPAC, and HVA in the corpus striatum, hypothalamus, and lateral olfactory tract regions, however, returned to nearly control levels 60 days following the end of styrene treatment (Table 4).

Dopamine Uptake and Release In Vitro

The effects of styrene and styrene oxide on DA uptake and release were measured in vitro in rat striatal synaptosomes, and the results are presented in Table 5. Based on 100% stimulation by tyramine with respect to release of DA, it is seen that both styrene and styrene oxide produced significant stimulation of DA release almost similar to that due to tyramine, suggesting that both styrene and styrene oxide are highly capable of inducing DA release from striatal synaptosomes. On the other hand, both styrene and styrene oxide did not cause any significant change in the uptake of DA in striatal synaptosomes, as evident by relatively low percentage (nonsignificant) of inhibition of DA uptake when compared to that by mazindol.

Binding Study

Neither the B_{max}, i.e., maximum number of binding sites nor the affinity of the receptor K_D (obtained from Scatchard plots) for specific ^{3}H-spiroperidol binding to corpus striatal membrane was significantly altered 7 and 30 days after the termination of subchronic treatment with styrene when compared with the control values. B_{max} control = 294 ± 14.8, B_{max} treated = 358 ± 32, and 302 ± 19 pmol bound per gram of protein, K_D control = 0.85 ± 0.05 nm, and K_D treated = 0.77 ± 0.04 and 0.72 ± 0.08 nM at two different time points (mentioned above) following the end of styrene treatment.

Tyrosine Hydroxylase Activity (TH Activity)

The results of tyrosine hydroxylase activity by L-DOPA accumulation after decarboxylase inhibition are presented in Table 6. No differences are apparent in the ability of the DA agonist to inhibit TH activity in styrene-treated compared to control animals in either brain region. TL-99 significantly antagonized the GBL effect in both exposure groups. Neither were significant differences in L-DOPA concentrations observed in treated and control rats after NSD1015 administration alone or in combination with GBL.

Tests of Motor Function

Significant depressions in motor function were noted in the high-dose (0.5 g/kg/day)-treated animals when compared

TABLE 4

LEVELS (μg/g) OF DOPAMINE AND ITS PRINCIPAL METABOLITES IN DIFFERENT BRAIN REGIONS 60 DAYS FOLLOWING THE END OF SUBCHRONIC TREATMENT WITH STYRENE (0.5 g/kg/DAY)

Dompamine and its metabolites	Control	Treated (0.5 g styrent/kg/day)
	Corpus Striatum	
DA	11.27 ± 0.95*	9.87 ± 1.00*
DOPAC	1.72 ± 0.13	1.58 ± 0.15
HVA	1.45 ± 0.12	1.35 ± 0.11
	Lateral Olfactory Tract	
DA	2.66 ± 0.20	2.32 ± 0.22
DOPAC	0.48 ± 0.04	0.43 ± 0.04
HVA	0.25 ± 0.02	0.21 ± 0.02
	Hypothalamus	
DA	0.85 ± 0.07	0.78 ± 0.06
DOPAC	0.34 ± 0.03	0.29 ± 0.03
HVA	0.19 ± 0.02	0.15 ± 0.01

*Results are averages ± SE of six rats.

TABLE 5

THE EFFECTS OF STYRENE AND STYRENE OXIDE ON DOPAMINE (DA) UPTAKE AND RELEASE IN RAT STRIATAL SYNAPTOSOMES IN VITRO

	DA Uptake		DA Release	
Treatment	Tissue DA (nmol/mg)	Inhibition % mazindol	Tissue DA (nmol/mg)	Stimulation % tyramine
Control	0.82 ± 0.05*	—	0.73 ± 0.04	—
Mazindol, 100 μM	0.50 ± 0.03†	100	—	—
Tyramine, 100 μM	—	—	0.45 ± 0.03†	100
Styrene, 100 μM	0.72 ± 0.05	31.3	0.50 ± 0.03†	82.1
Styrene oxide, 100 μM	0.74 ± 0.06	30.46	0.51 ± 0.04†	78.6

*The results are averages ± SE of four separate experiments, involving four separate pools of striatal tissue, each from 10–15 rats used. For details, see the Methods section.

†Significantly different from control, $p < 0.05$.

with the control values (Fig. 2 and Fig. 3). By 56 days of treatment with styrene, motor activity counts differed significantly between the high-dose-treated group and the control group, $F(2, 27) = 3.37$, $p < 0.05$ (Fig. 2). Similarly, motor activity counts differed significantly between the high-dose-treated group and the control group on days 70, $F(2, 27) = 4.89$, $p < 0.025$, and 84, $F(2, 27) = 7.84$, $p < 0.005$, during styrene treatment. The difference between the high-dose-treated group and the control group in the landing foot-spread started to be significant on day 56, $F(2, 27) = 3.38$, $p < 0.05$, and continued to be significant on day 70, $F(2, 27) = 3.57$, $p < 0.05$, and day 84, $F(2, 27) = 4.39$, $p < 0.025$, during styrene treatment (Fig. 2). Similarly, significant decreases in motor activity and motor coordination were noticed in the 0.5 g/kg/day styrene-treated group compared to the vehicle control on days 1, 7, 15, or 30 following termination of styrene treatment (Figs. 4 and 5). Thus, significant decreases in motor activity counts occurred on day 1, $F(2, 27) = 7.97$, $p < 0.005$, day 7, $F(2, 27) = 6.67$, $p < 0.005$, day 15, $F(2, 27) = 6.54$, $p < 0.005$, and day 30, $F(2, 27) = 4.27$, $p < 0.025$, compared with those in the control group (Fig. 4). However, such decrease in motor activity became nonsignificant 45 days, $F(2, 27) = 1.14$, $p > 0.05$, after the end of styrene treatment (Fig. 4). Similarly, the difference between the high-dose–treated group and the control group in the landing foot-spread was significant on days 1 ($F = 4.99$, $p < 0.025$), 7 ($F = 4.83$, $p < 0.025$), 15 ($F = 4.77$, $p < 0.025$), and 30 ($F = 3.61$, $p < 0.05$) following the end of styrene treatment (Fig. 4). On the other hand, such difference was no longer significant ($F = 0.88$, $p > 0.05$) from 45 days following the end of styrene administration (Fig. 4).

Subchronic treatment of rats with a higher dose (0.5/g/kg/day) of styrene resulted in significant motor incoordination during such treatment (Fig. 3). Thus, relative stride length was significantly shortened compared to those in control group on day 70, $F(2, 27) = 4.35$, $p < 0.025$, and day 84, $F(2, 27) = 5.69$, $p < 0.01$, during subchronic treatment with the higher dose of styrene (Fig. 3). Furthermore, relative stride width was significantly increased on day 56, $F(2, 27) =$

TABLE 6

TYROSINE HYDROXYLASE ACTIVITY IN STYRENE-TREATED AND CONTROL RATS AS DETERMINED BY L-DOPA ACCUMULATION

	Striatum		Hypothalamus	
Drug treatment	Control	Styrene-treated (0.5 g/Kg)	Control	Styrene-treated (0.5 g/Kg)
NSD1015*	212.6 ± 25.1†	233.7 ± 26.5	136.9 ± 12.5	149.5 ± 16.2
GBL + NSD1015	286.3 ± 32.5	255.6 ± 36.1	274.2 ± 43.5	261.3 ± 35.4
GBL + NSD1015 + TL-99 (5 mg/kg)	139.8 ± 8.4	143.2 ± 9.3	125.6 ± 8.7	136.2 ± 9.4
(10 mg/kg)	158.7 ± 11.3	147.5 ± 10.6	132.5 ± 6.8	139.1 ± 9.1

*NSD1015 was administered to produce an accumulation of L-DOPA as an index of enzyme activity; endogenous L-DOPA levels could not be detected without this agent.

†The enzyme activity was determined 24 h after the end of the subchronic treatment with a higher dose (0.5 g/kg) of styrene.

Values are expressed as means ± SE in picomoles L-DOPA/mg protein for five separate experiments.

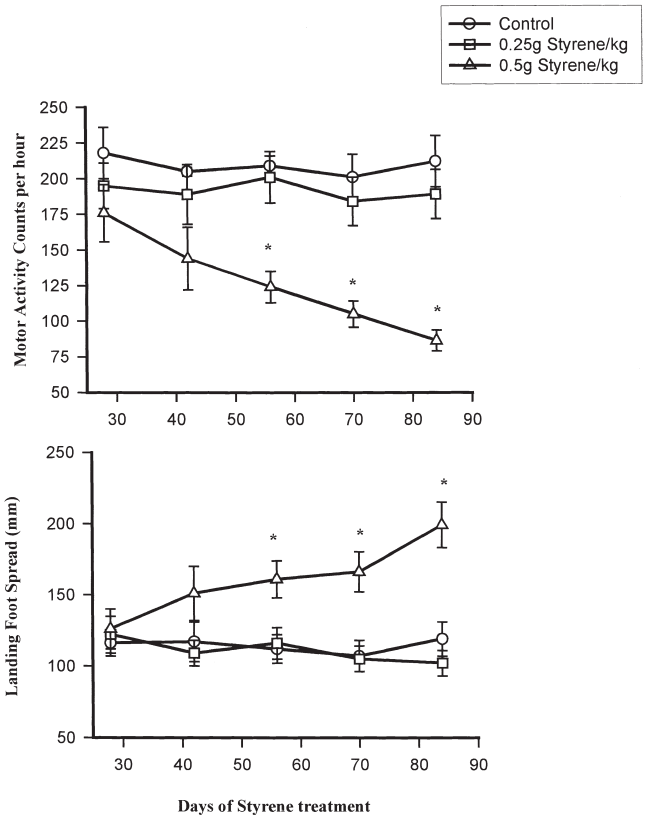

FIG. 2. Motor activity and landing foot-spread behavior at different days during 13-week of styrene treatment. All values are the means ± SE of 10 rats in each group. *Indicates significantly different from corresponding control, $p < 0.05$.

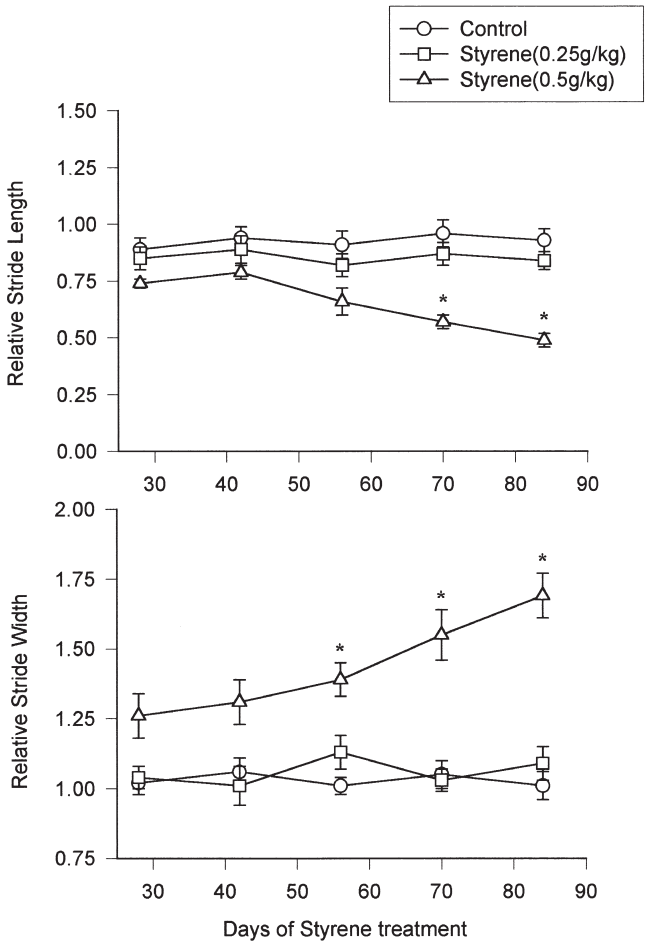

FIG. 3. Relative stride length and relative stride width behavior at different days during 13-week of styrene treatment. *Indicated significantly different from corresponding control, $p < 0.05$.

4.66, $p < 0.025$, on day 70, $F(2, 27) = 6.59$, $p < 0.005$, and on day 84, $F(2, 27) = 11.17$, $p < 0.001$, during 13 weeks of styrene treatment with a higher dose (Fig. 3). The widened gait reflected their inability to support their hindquarters. They were unable to position their hind legs properly when dropped, and this was reflected in a relative hindlimb foot spread that was markedly wider than both vehicle- and 0.25 g/kg/days styrene-treated groups. However, such a significant increase in motor incoordination disappeared within 45 days following the end of styrene administration (Figs. 4 and 5). Thus, animals receiving the high dose of styrene (0.5 g/kg/day) were significantly less active in motor function. Therefore, the effect of such subchronic treatment was to decrease the locomotor activity. Similarly, significant decreases in motor coordination were also noticed in the high-dose–treated group compared to vehicle control on days 1, 7, and 30 following the end of styrene treatment (Fig. 5). Thus, significant decreases in relative stride length occurred on day 1, $F(2, 27) = 9.27$, $p < 0.001$, day 7, $F(2, 27) = 5.88$, $p < 0.01$, and on day 30, $F(2, 27) = 4.63$, $p < 0.025$, following the end of styrene treatment when compared with those in the control group (Fig. 5). Significant increases in relative stride widths also occurred on day 1, $F(2, 27) = 10.15$, $p < 0.001$, day 7, $F(2, 27) = 5.53$, $p < 0.01$, and on day 30, $F(2, 27) = 5.96$, $p < 0.007$, following the termination of styrene administration when compared with those in the control group (Fig. 5). However, the relative stride length or the relative stride width due to high-dose-styrene-treated rats was no longer significantly different from that of control group within 45 days after the end of styrene treatment (Fig. 5).

DISCUSSION

The present results have shown that styrene treatment alters dopaminergic function in certain brain regions that are important in the initiation and control of movement (movement disorders). Therefore, based on time studies, it is speculated that the regional changes in brain contents of dopamine and its metabolites due to a higher dose of styrene may be responsible to alter the motor function in rats. However, the deficits in motor function resulting from styrene exposure were not permanent and recovered to control levels within 45 days following cessation of such treatment. This time-dependent improvement in motor performance indicates that subchronic exposure to styrene does not cause an irreversible depletion of dopamine from intact neurons. The recovery of motor function in rats observed from 45 days following the end of styrene treatment may result at least partially from a compensatory increase in dopamine turnover by remaining neurons in three different brain areas studied. This is quite evident from the results obtained for dopamine and its principal metabolites 45 days after the end of styrene treatment

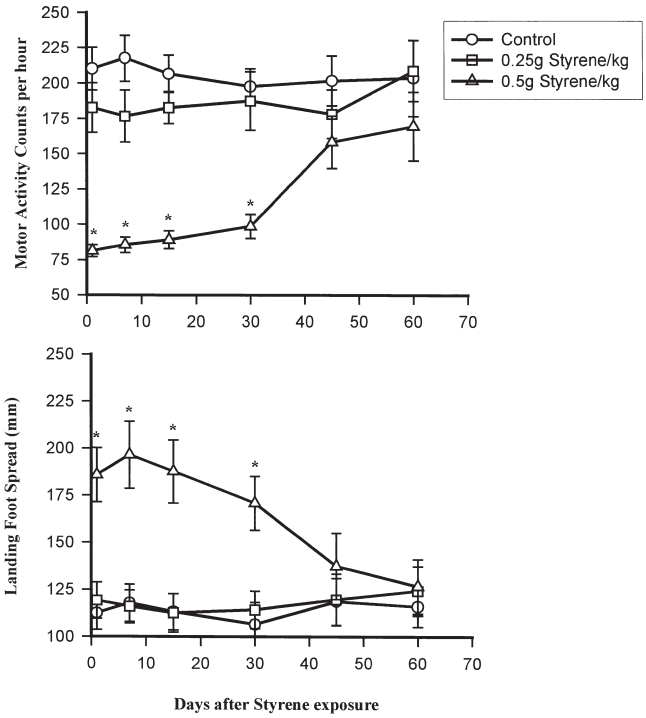

FIG. 4. Motor activity and landing foot-spread behavior at different days after termination of 13-week of styrene treatment. *Indicates significantly different from corresponding control, $p < 0.05$.

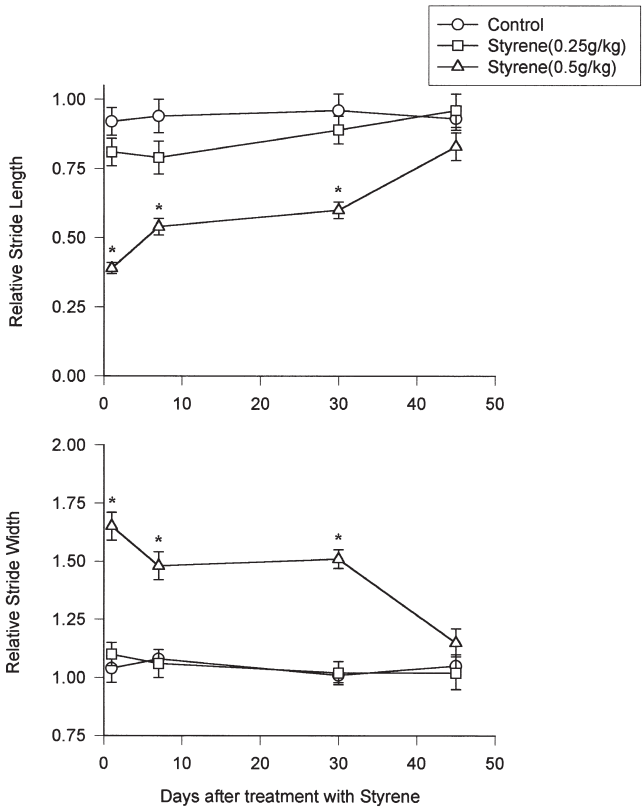

FIG. 5. Relative stride length and relative stride width behavior at different days after termination of 13-week of styrene treatment. *Indicates significantly different from corresponding control, $p < 0.05$.

(Table 4). Such behavioral recovery agrees with those reported in styrene-exposed workers (24). Epidemiological studies have shown that neuropsychological deficits such as slowing of reaction time, loss of color vision, and vestibulooculomotor dysfunction are reliably induced by styrene exposure at levels near or below current exposure standards, ranging from 20–50 ppm. However, despite the workers exposed for many years, no indications of persisting damage to the nervous system were noticed (24).

The present results are in contradiction with the results reported by Husain et al. (15), who showed increased levels of serotonin and norepinephrine but without any effect on dopamine levels in the whole brain of adult male albino rats treated orally with 0.91 g/kg b.wt. of styrene for 15 days. Whether such difference is due to difference in the duration of treatment, and/or different strain of animal species is not known at this time. However, these authors have shown later that repeated administration caused a significant increase in the specific binding of ^{3}H-spiroperidol to striatal membranes 24 h after the end of the administration. The increase in binding may be due to changes in the affinity of receptor sites or increase in the number of receptors (1). The present results are, however, in agreement in parts with those reported by Mutti et al. (20,21), who showed dose-dependent depletions in striatal and tubero-infundibular dopamine contents, but are in disagreement with other results showing increases in homovanillic acid contents in the same brain areas despite an inhibition of tyrosine hydroxylase activity in rabbits. Norepinephrine levels were, however, unaffected in agreement with our results.

Neurotransmitter metabolite concentrations are thought to represent functional transmitter release and degradation in conjunction with intraneuronal deamination of the newly synthesized transmitter (8). Thus, the altered regulation of dopamine content and its metabolites in response to styrene is likely due to styrene-induced changes in vesicular storage or release of a neurotransmitter. Indeed, styrene caused a release of synaptosomal dopamine similar to that of tyramine. Thus, the decreased DOPAC and HVA contents in certain brain regions observed in this study are consistent with this hypothesis. Thus, the neurotoxic action of styrene on dopaminergic neurons may be primarily presynaptic in nature, not related to impaired regulation of DA synthesis.

Because the effect of subchronic styrene treatment on L-DOPA accumulation induced by GBL administration alone did not attain statistical significance, the results suggest that autoreceptor-mediated regulation of the enzyme is unperturbed, other factors, such as the subchronic effects of styrene on vesicular storage of transmitter, cannot be ruled out. Thus, under conditions of subchronic treatment, styrene-induced impairments in measures of dopaminergic activity cannot be attributed to altered regulation of TH activity.

Although the present results are mainly discussed by considering a presynaptic action of styrene, a possibility of postsynaptic effects of styrene should also be considered. On this line, it has been shown that repeated IP treatment of male rats with 25 and 50 mg/kg of styrene oxide (which are much lower doses than those in the present study) for 14 days results in an increase in total number of striatal dopamine re-

ceptor binding sites (B_{max}) without any significant change in the affinity of the receptor (K_D). This suggests a denervation type of supersensitivity of dopamine receptor. However, the effect of such treatment with styrene oxide on the dopamine receptor appears to be reversible, as no significant change in ^{3}H-spiroperidol binding could be detected between control and treated groups 14 days after cessation of such treatment (37). Based on our present study, specific [^{3}H] spiroperidol binding measured in vitro was unaltered 7 or 30 days after styrene treatment. This implies that despite the neuronal loss induced by styrene in rats, dopaminergic transmission was not reduced to a level that would result in an overall development of dopamine receptor supersensitivity in the striatum.

Collectively, these studies indicate that the neurotoxic action of styrene due to subchronic exposure is primarily presynaptic in nature, and suggest at least a partial dependence on impaired regulation of DA content and increased DA release. Further studies are required to isolate the synaptic mechanisms that underlie these observations.

ACKNOWLEDGEMENTS

Portions of this work were presented at the 34th Annual Meeting of the Society of Toxicology, 1995, Baltimore, MD. This work was supported in parts by the Institut de recherche en santé et en sécurité du travial du Québec, and by CAFIR, Université de Montréal. The technical assistance and advice from Neuropsychology Department, Université de Montréal is gratefully acknowledged. The technical assistance of Mrs. S. Yamaguchi and C. Denniel as well as the expert secretarial assistance of Chantal Bélisle are also gratefully acknowledged.

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