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Toluene Induces Behavioral Activation Without Affecting Striatal Dopamine Metabolism in the Rat: Behavioral and Microdialysis Studies

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KONDO, H., J. HUANG, G. ICHIHARA, M. KAMIJIMA, I. SAITO, E. SHIBATA, Y. ONO, N. HISANAGA, Y. TAKEUCHI AND D. NAKAHARA. *Toluene induces behavioral activation without affecting striatal dopamine metabolism in the rat: Behavioral and microdialysis studies.* PHARMACOL BIOCHEM BEHAV 51(1) 97-101, 1995.—We examined the effects of toluene on the release of dopamine (DA) and its metabolites in rat striatum using microdialysis. Intraperitoneal injection of 800 mg/kg toluene significantly increased motor activity in rats, as did methamphetamine (MAP) (1 mg/kg). However, 800 mg/kg toluene did not affect the extracellular levels of DA, 3,4-dihydroxyphenylacetic acid, homovanillic acid, or 5-hydroxyindoleacetic acid. This is in contrast to MAP, which significantly increased extracellular DA and decreased the extracellular levels of its metabolites. These results suggest that toluene-induced behavioral augmentation may not be associated with alterations in DA or serotonin neurochemistry such as are associated with MAP-induced behavioral augmentation.

Toluene Dopamine Motor activity Striatum Methamphetamine Microdialysis

THE ORGANIC solvent toluene is widely used in industry. Exposure to toluene causes changes in the CNS, but the exact mechanism is not known. Recent studies have indicated that treatment with toluene can change central monoamine neurotransmission. The literature, however, is inconsistent. Kiriu et al. (15) reported that the inhalation of toluene decreased noradrenaline (NA) levels in the dorsal part of rat pons, an area containing the locus coeruleus where the NA cells are located. They found, moreover, that such inhalation decreased dopamine (DA) levels in the hypothalamus and in the ventral part of the midbrain, containing the substantia nigra in which many DA cells are located. Thus, they suggested that toluene decreases catecholamine turnover in the brain. On the other hand, other researchers have reported inconsistent effects in tissue levels of neurotransmitters after subacute and long-term treatment with toluene: no effect on DA levels (21); lowered serotonin (5-HT) levels and increased NA levels (1); increased NA, DA, and 5-HT levels (9); increases in DA and glutamine, and a decrease in acetylcholine (ACh) (10); and decreases in NA and DA (11). Furthermore, no studies have evaluated the effects of exposure to toluene on neurotransmitter release at the nerve terminals.

Recently, microdialysis was devised as a brain perfusion technique and has been widely used to measure extracellular neurotransmitter levels in the brain. The use of this technique has made it possible to analyze specific neural activities in awake and freely moving animals (24). Microdialysis has been used, for example, to correlate alterations in neurotransmit-

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ters with behavioral changes induced by monoaminergic drugs, such as amphetamine (4,5,17) or cocaine (2,4,5).

In the present study, we used microdialysis to examine the effects of acute treatment with toluene on monoamine release, particularly dopamine release, in rat striatum, and also measured motor activity. These changes were compared to those induced by treatment with methamphetamine (MAP), a compound known to release dopamine (7,24).

MATERIALS AND METHODS

Animal Surgery

Male Wistar rats weighing 200–300 g were used. Animals were anesthetized with sodium pentobarbital [50 mg/kg, intraperitoneally (IP)] and were stereotaxically implanted with a 22-ga guide cannula in the left striatum (STR) at A: 1.0 mm, L: 3.1 mm from bregma, and 4.5 mm from the skull. The coordinates were chosen according to the stereotaxic atlas of Paxinos and Watson (25). Following implantation, the guide cannula was firmly fixed to the skull with two anchor screws and dental cement, and a dummy probe was inserted into the guide. The dummy probe was left in the brain until the dialysis experiment was started.

After the end of the experiment, the animals were killed under deep anesthesia and brains were removed and placed in 10% formalin-saline for 7 or more days. The location of the probe was verified by visual inspection under a dissecting microscope.

Brain Dialysis

The dialysis experiment was carried out as described previously (22,23). Twenty-four to 48 h after surgery, the dummy probe was removed from the guide cannula and a dialysis probe was gently inserted into the guide, to which it was secured using sticky wax. The microdialysis probe was concentric in design with side-by-side inlet and outlet arrangement, fitted with a 4-mm length of dialysis tubing (0.23 mm in diameter, mol. wt. cutoff of 35,000; Nikkiso, Japan). The probe within the STR was perfused with Ringer's solution (147 mM NaCl, 4 mM KCl, and 1.2 mM $CaCl_2$ at pH 7.0) at a constant flow rate of 2 ml/min. Following a 2-h stabilization period, three samples were collected at 20-min intervals to determine basal levels of monoamine and its metabolites. Immediately after this, rats were administrated toluene or methamphetamine (MAP) IP and successive 20-min samples were again collected for 4–5 h.

Injection of Toluene and MAP

After the baseline of monoamine and its metabolites was determined, rats were injected intraperitoneally with 80 (n = 5), 250 (n = 6) or 800 (n = 7) mg/kg toluene. Toluene was dissolved in olive oil and injected in volumes of 8 ml/kg. Control rats were injected with the corresponding vehicles (n = 6). Rats injected with MAP (1 mg/kg, dissolved in saline: n = 6) served as the positive controls.

HPLC Assay of Dialysate

Levels of DA and its major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the dialysates were determined by HPLC with electrochemical detection (HPLC-ECD) as described previously (22,23). The HPLC system consisted of an EICOM EP-10 pump, an ECD-100 electrochemical detector with a WE-3G graphite working electrode, a reversed-phase Eicompack MA-ODS column (4.6 × 250 mm, 7 μm; EICOM; Japan) and a Shimadzu Chromatopack CR-5A integrator. A 70-mM citric acid and 100-mM sodium acetate buffer (pH 4.0) containing 1.01 mM sodium 1-octanesulfonate, 26.9 μM ethylenediaminetetraacetic acid, and 13% methanol (v/v) served as the mobile phase. The instrument settings were: flow rate 1.2 ml/min, detector potential +650 mV, sensitivity 2 nA/V full scale, and sample volume 35 μl.

Measurement of Motor Activity

Each rat was individually placed in a transparent acrylic box (30 × 30 × 35 cm) that was located at the center of the animal activity apparatus with magnet-field coils (Automex II; Columbus INS, Columbus, OH). Motor activity was measured simultaneously with microdialysis.

Statistical Analysis

Neurochemical data were expressed for each rat as the percent change from the mean of the three 20-min samples taken before injection of toluene or MAP. All data are shown as mean ± SE. A two-way repeated-measures analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons, was used, but the ANOVA was performed after log-transformation in case of skewed distributions.

RESULTS

Animal Behavior

As shown in Fig. 1, motor activity significantly increased following IP injection of 800 mg/kg toluene and 1 mg/kg MAP ($p < 0.01$). Immediately after IP injection of 800 mg/kg toluene, rats showed tachypnea and became excited. Five to 10 min after this, the animals commenced sniffing and repetitive head movements and soon started racing around the cage enclosure, exhibiting an ataxic gait and frequently lying on their bellies or pawing their sides. Rats continued moving about the cage for 2–3 h. Behavior induced by 1 mg/kg MAP

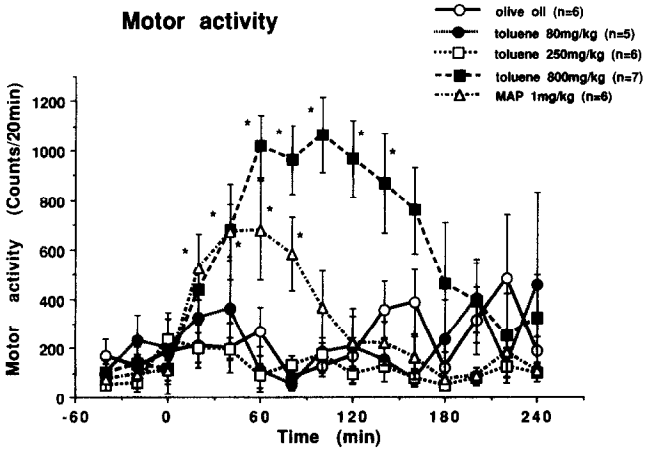

FIG. 1. Effects of toluene and methamphetamine (MAP) on locomotor activity in rats. Rats were injected intraperitoneally with toluene (80, 250, and 800 mg/kg), MAP (1 mg/kg, positive control), and olive oil (vehicle, control). *$p < 0.05$ as compared to respective control values using Dunnett's test.

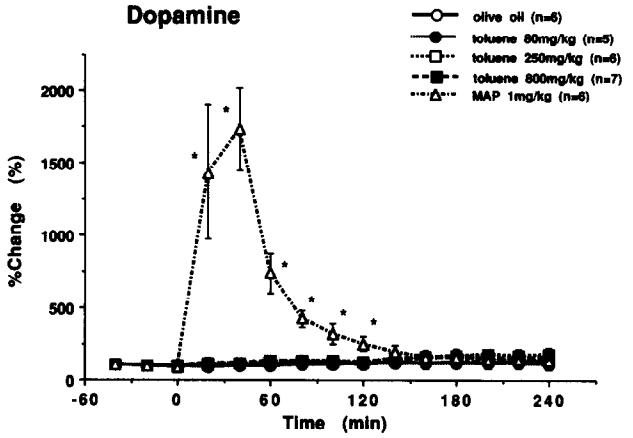

FIG. 2. Effects of toluene and methamphetamine (MAP) on extracellular dopamine level in rat striatum. Rats were injected intraperitoneally with toluene (80, 250, and 800 mg/kg), MAP (1 mg/kg, positive control), and olive oil (vehicle, control). *$p < 0.05$ as compared to respective control values using Dunnett's test.

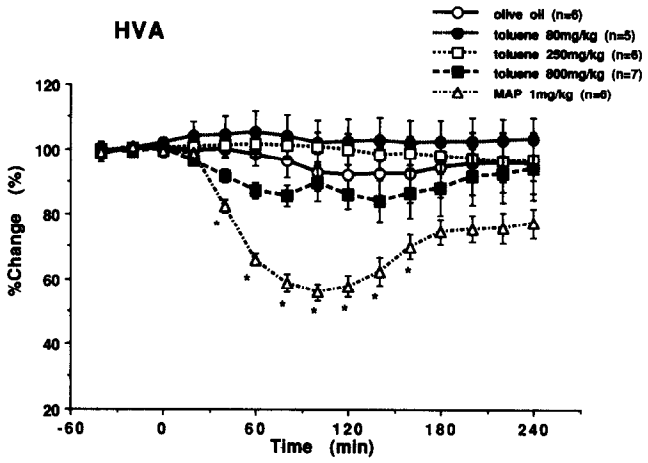

FIG. 4. Effects of toluene and methamphetamine (MAP) on extracellular homovanillic acid (HVA) level in rat striatum. Rats were injected intraperitoneally with toluene (80, 250, and 800 mg/kg), MAP (1 mg/kg, positive control), and olive oil (vehicle, control). *$p < 0.05$ as compared to respective control values using Dunnett's test.

resembled that following 800 mg/kg toluene except that no ataxia was seen. In rats injected with toluene, however, locomotion was more prominent than stereotypy, which was the converse of the 1-mg/kg MAP-induced behavior. At doses of 80 and 250 mg/kg, toluene had little effect on rat behavior.

Accumulation of DA and Metabolites

Figures 2–5 show the results for extracellular levels of striatal DA, DOPAC, HVA, and 5-HIAA, respectively. The basal values, the mean of three 20-min dialysates taken before injection of toluene or MAP, were as follows (mean ± SE, n = 30): DA, 35.6 ± 2.9 fmol/20 min; DOPAC, 31.3 ± 1.8 pmol/20 min; HVA, 23.2 ± 1.3 pmol/20 min; 5-HIAA, 21.4 ± 1.2 pmol/20min. MAP (1 mg/kg) induced a rapid and significant increase in DA ($p < 0.01$) and a decrease in the levels of DA metabolites, DOPAC ($p < 0.01$), and HVA ($p < 0.01$). By contrast, at any dose of toluene, no significant change was measured in the levels of DA, DOPAC, and HVA in rat striatum. Neither MAP nor toluene affected the 5-HIAA level ($p = 0.45$).

DISCUSSION

Inhalation exposure to toluene enhances locomotion at low to intermediate concentrations (< 2000 ppm) but decreases motor activity at higher concentrations (> 2000 ppm) in rats and mice (8,16,28,33). Also, it has been reported that toluene-exposed rats initially became excited and moved about the cage, and later became calm. During the period of toluene exposure, these animals also exhibited ataxia, resting tremor,

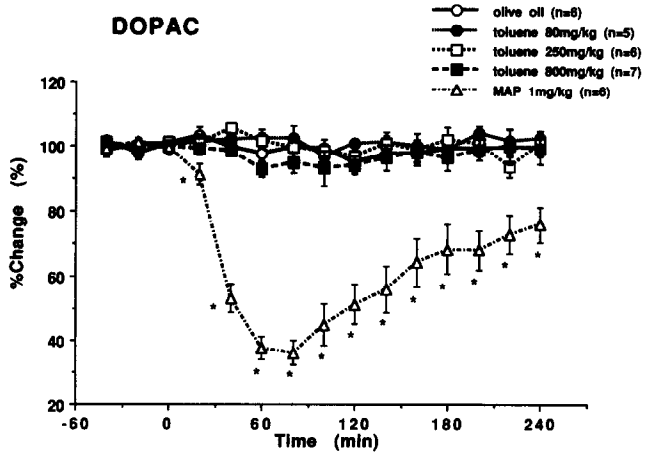

FIG. 3. Effects of toluene and methamphetamine (MAP) on extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) level in rat striatum. Rats were injected intraperitoneally with toluene (80, 250, and 800 mg/kg), MAP (1 mg/kg, positive control), and olive oil (vehicle, control). *$p < 0.05$ as compared to respective control values using Dunnett's test.

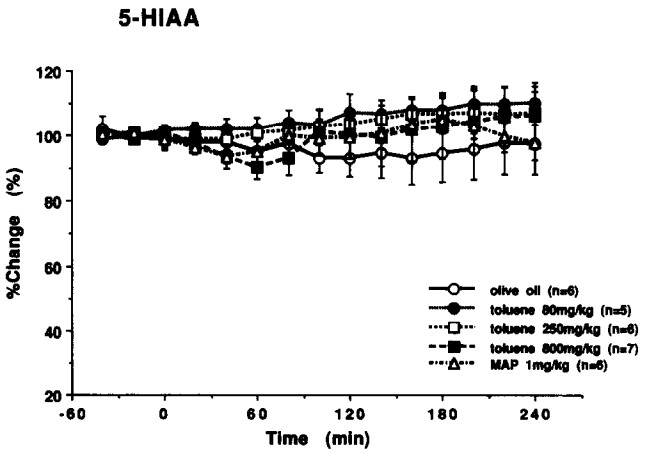

FIG. 5. Effects of toluene and methamphetamine (MAP) on extracellular 5-hydroxyindoleacetic acid (5-HIAA) level in rat striatum. Rats were injected intraperitoneally with toluene (80, 250, and 800 mg/kg), MAP (1 mg/kg, positive control), and olive oil (vehicle, control).

head weaving, hindlimb abduction, and foot twitching (27,34). Consistent with the previous results, rats injected with 800 mg/kg toluene in the present study exhibited enhanced locomotion with sniffing and head movements, and ataxic gait. Moreover, the behavioral profiles induced by 800 mg/kg toluene closely resembled those following 1 mg/kg MAP. Previous studies have demonstrated that the behavioral augmentation produced by amphetamine (17) or cocaine (14,26) is associated with alterations in DA and 5-HT neurochemistry. It is well known that drugs abused by humans, such as amphetamine (4,5,17), cocaine (2,4,5), phencyclidine (5,29), ethanol (4,5,12,32), and nicotine (4,5,13), preferentially increase extracellular DA concentrations in the brain. This led us to postulate that toluene, a drug of abuse, might increase extracellular DA levels in rat striatum. But our results demonstrated no effect of acute toluene administration on striatal dopamine metabolism. Thus, our results clearly showed that the 800-mg/kg toluene-induced behavioral augmentation was not associated with striatal DA release. This contrasts with measurements made during the amphetamine (17) and cocaine (3,14) -induced behavioral responses, which implicate the nigrostriatal, mesolimbic, and mesocortical DA systems.

In view of the increased motor activity induced by 800 mg/kg toluene, we suspect that toluene must cause some change in central neurotransmission. One possibility is that toluene might affect the mesocorticolimbic DA system, which was not examined in the present study but appears to play an important role in the control of spontaneous locomotor behavior (3,14,17). Another possibility is that toluene might affect animal behaviors via non-DA pathways. It is also possible that toluene might affect directly postsynaptic DA or other receptors without any change in the extracellular concentrations of neurotransmitters. It has been previously suggested from in vitro work that toluene alters the function of brain membranes, such as membrane lipid composition (27) or membrane fluidity (30), and that this leads to a reduction in D_2 (31) and 5-HT (34) receptor affinity. However, recent studies (6,31) showed no in vivo effect of toluene on membrane fluidity. Finally, toluene induces reactive oxygen species (ROS) in the brain (18–20). Alterations of cellular membrane functions by ROS damage may change rat behavior. Further investigation is therefore needed for a full understanding of the neuronal mechanism underlying toluene neurotoxicity.

In conclusion, the present study demonstrated that toluene-induced behavioral augmentation may not be associated with alterations in DA neurochemistry. This contrasted with the effect of methamphetamine, which increased extracellular DA concentrations while inducing behavioral activation.

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