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Acute Toluene Induces Biphasic Changes in Rat Spontaneous Locomotor Activity Which Are Blocked by Remoxipride

ARTHUR C. RIEGEL AND EDWARD D. FRENCH

University of Arizona, Health Sciences Center, Department of Pharmacology, School of Medicine, Tucson, AZ 85716

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RIEGEL, A. C. AND E. D. FRENCH. *Acute toluene induces biphasic changes in rat spontaneous locomotor activity which are blocked by remoxipride*. PHARMACOL BIOCHEM BEHAV **62**(3) 399–402, 1999.—The behavioral hyperactivity elicited by most drugs of abuse has been linked to changes in mesolimbic dopamine neurotransmission. However, the locomotor stimulant effects of toluene, a constituent in many abused inhalants, has not been clearly associated with this site of action. The present study was designed to examine the hypothesis that toluene-induced hyperactivity is also dependent upon intact dopamine neurotransmission. Using photocell-equipped cages, 600–1200 mg/kg toluene produced an inverted U-shaped dose response. However, in the presence of 5 mg/kg remoxipride, a selective D_2-dopamine antagonist toluene-induced hyperactivity was reduced by 57%. The effects of remoxipride appear to be selective as a pretreatment, as it did not reduce either spontaneous locomotor activity or the stimulatory effects of the muscarinic antagonist scopolamine. These results clearly show that toluene induces locomotor hyperactivity through a dopamine-dependent mechanism. Because the mesolimbic dopamine system has been shown to play a role in the rewarding properties of drugs of abuse, its activation by toluene may also underlie the abuse potential of this and other inhalants. © 1999 Elsevier Science Inc.

Toluene Remoxipride Scopolamine Locomotor activity Mesolimbic dopamine Inhalant abuse Solvents Volatile substance abuse

THE recognition of inhalant abuse (also known as volatile substance abuse) as a significant health problem (12) has provided a stimulus to investigate the neuronal mechanisms by which such substances affect CNS neurotransmitter processes. It is well known that the abuse of inhalants, of which toluene appears to be the major psychoactive constituent, by humans produces a variety of symptoms including euphoria, hallucinations, dependence, psychosis, and gross neurological damage [for review, see (3)].

Although acute toluene exposure alters a variety of central neurotransmitter levels (acetylcholine, GABA, dopamine) with a large degree of variability depending on dose and brain region, no systematic studies have been carried out to definitively relate changes in locomotor activity to alterations in a specific neurotransmitter [for review, see (1)]. Nevertheless, it seems a plausible hypothesis that dopamine would be involved, because there is a large amount of evidence showing that changes in spontaneous locomotor activity following the administration of psychomotor stimulants are mediated, in part, by the mesolimbic DA system originating in the midbrain ventral tegmentum (5). Moreover, it is this system whose activation is also linked to the potent rewarding effects of abused drugs (8,11). Indeed, it has been shown that nonhuman primates will lever press for exposure to toluene vapor, suggesting the potential for addiction (18).

The present study was designed to assess the effects of toluene on spontaneous locomotor activity, and to determine whether these effects are sensitive to pretreatment with the D_2 selective DA antagonist remoxipride (14).

Requests for reprints should be addressed to E. D. French, University of Arizona, Health Sciences Center, Department of Pharmacology, School of Medicine, Tucson, AZ 85716.

METHOD

Animals

All experiments were conducted on male Sprague–Dawley rats (Harlan Sprague–Dawley, Inc.) weighing between 240–300 g. The animals were maintained in a central animal facility under controlled lighting (12 L:12 D schedule) and temperature, with free access to food and water.

Drugs

Toluene (HPLC grade, 99.8% purity, Baxter Corporation) was administered in an olive oil vehicle with a total injection volume <1.5 ml. Scopolamine HCl (Sigma Chemical Co.) and remoxipride HCl (Astra) were dissolved in saline, and doses were calculated based upon weight of the salts. All compounds were injected intraperitoneally.

Measurement of Locomotor Activity

Locomotor activity was measured in cages with wire mesh (10 × 10 mm) floors, fronts, and backs, and clear plastic tops. Each cage measured 20 cm high × 25 cm wide × 36 cm long, with two infrared photocell beams placed 2 cm above the floor and dividing the long axis of the cage into three equal segments. Photocell-beam interruptions were wired to electronic counters and were recorded every 20 min. All testing took place in a quiet, moderately lighted room with opportunity for behavioral observations following drug administration. For several days prior to drug treatments all rats were acclimated to the room and test cages for a minimum of 5 h. On the day of injection each rat was again exposed to the testing cage for an additional 90 min. Following injection, no entry into the room occurred until the 3-h test period was complete. Gross behavioral observations were noted but not quantitated.

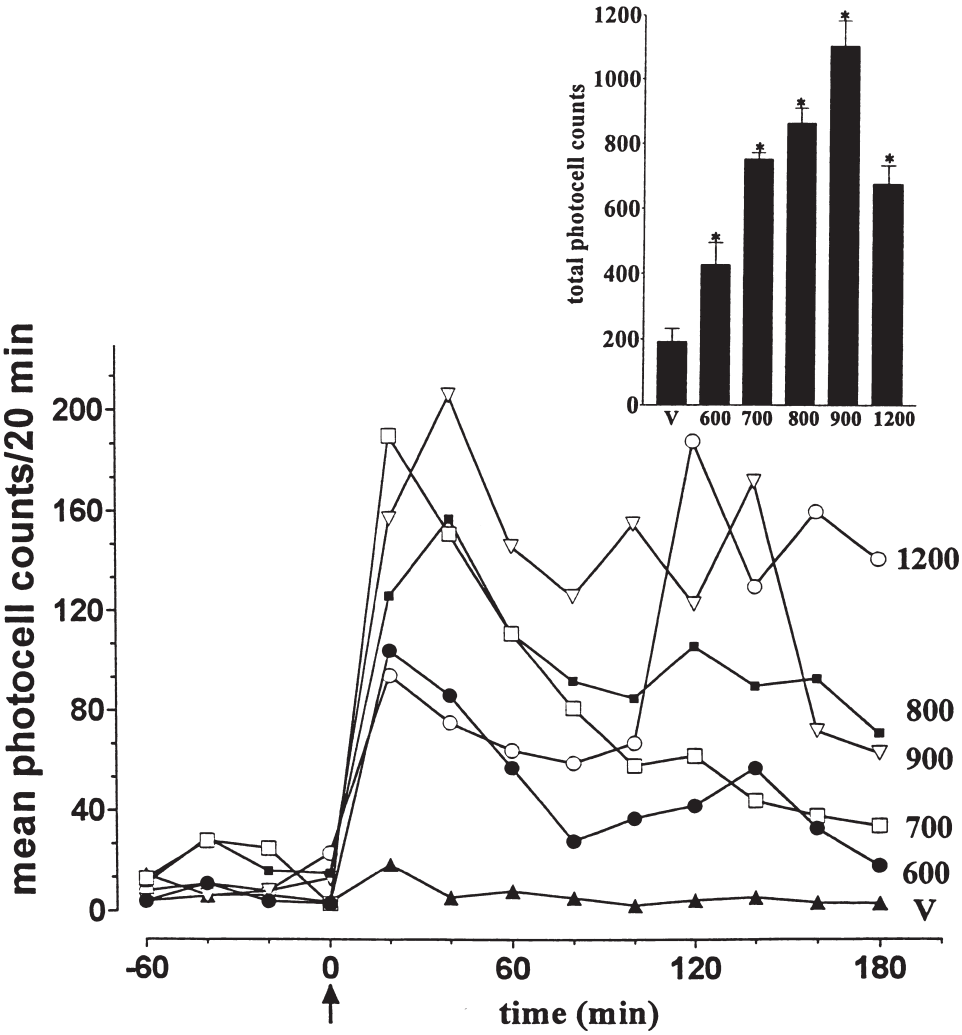

FIG. 1. Dose-dependent effects of toluene on rat locomotor activity. Bottom portion of figure shows time course of effects following intraperitoneal injections of vehicle (V) (open bar) or toluene (arrow). Numbers to the right of the traces indicate dose of toluene administered. Upper bar graph shows total cumulative photocell counts over the 3-h recording period following vehicle or toluene (numbers as doses). *$p < 0.01$ vs. vehicle by *t*-test for group data. Each point is the mean response of five to seven animals.

RESULTS

Locomotor Activity

Intraperitoneal injections of toluene produced dose-related changes in locomotor activity. The four lowest doses of toluene markedly enhanced spontaneous activity in a concentration-related fashion when compared to rats injected with vehicle (191 ± 99 photocell counts/3 h, $n = 6$; data expressed as mean ± SEM). For example, toluene at 600, 700, 800, and 900 mg/kg ($n = 5$/dose) resulted in increases in photocell counts of 427, 654, 865, and 1106, respectively. These represent changes over control values of 123, 242, 352, and 479% (Fig. 1). In contrast, 1200 mg/kg toluene resulted in substantially fewer photocell interruptions (678 ± 186) over the recording period.

The locomotor time courses for the four lower doses also differed from the time course at the highest dose of toluene. For example, toluene at the 600–900 mg/kg range showed peak activity changes within 20–40 min postinjection, and roughly similar time courses for the decline of the drug effect (Fig. 1). However, the 1200 mg/kg dose caused a delayed onset of activity and only a slight decline in drug effect by the end of the 3-h recording period. Vehicle-treated animals showed a characteristic rapid decline in photocell counts following injection. The observation was also made that toluene at the four lower doses elicited increased grooming, sniffing, and jerky movements with variable rearing, until at the 1200 mg/kg dose the animals were ataxic and frequently fell to one side. This ataxia likely explains the delayed locomotor response and fewer total photocell counts. Because of the robust locomotor effect and minimal interference from ataxia, 900 mg/kg toluene was chosen for further testing with remoxipride.

Rats pretreated with remoxipride (5 mg/kg, IP) 20 min prior to toluene (900 mg/kg IP, $n = 9$) demonstrated significantly less forward directed locomotor behavior (471 ± 88 mean total counts, $n = 9$) (Fig. 2) than rats treated with 900 mg/kg toluene alone (1106 ± 71 mean total counts, $n = 9$). To control for possible nonspecific effects of remoxipride a group of animals received 1 mg/kg scopolamine following pretreatment with the D_2 antagonist or vehicle. Scopolamine appears to evoke hyperactivity through a dopamine-independent mechanism (7). In the scopolamine-challenged animals the resultant locomotor hyperactivity was virtually identical between the remoxipride (504 ± 72) and nonremoxipride treated groups (497 ± 88) (Fig. 1).

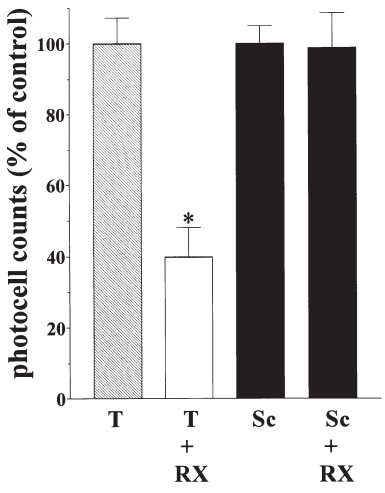

FIG. 2. Locomotor activity of toluene (T) and scopolamine (Sc) following pretreatment with the D_2-receptor antagonist remoxipride (Rx). *$p < 0.01$. Data averaged from five to nine animals in each treatment group.

The time courses for the remoxipride–toluene (900 mg/kg), vehicle–scopolamine, and remoxipride–scopolamine were very similar, with peak maximum activities occurring at 20–40 min followed by a rapid decline of drug effect, in direct contrast to the time course for the toluene 900 mg/kg alone (data not shown).

DISCUSSION

The present results argue that the ability of toluene to induce dose-dependent, biphasic alterations in spontaneous locomotor activity is likely related to increased DA neurotransmission, as this effect is significantly attenuated following blockade of D_2 dopamine receptors. Toluene at lower doses (600–900 mg/kg) steadily augmented locomotor activity, with an observable increase in the frequency of sniffing and rearing. Locomotor activity was most pronounced at the dose of 900 mg/kg. Earlier studies using single dosing paradigms also found robust hyperactivity with 800 mg/kg (10). Inhalation of toluene vapors in concentrations from 1000–7000 ppm have also been shown to increase locomotor activity (2,6,15,20). In contrast, toluene at the highest dose (1200 mg/kg) attenuated the spontaneous motor movement and produced behavioral signs of intoxication including ataxia, which apparently interfered with forward locomotion, thereby resulting in fewer total photocell interruptions. This effect is similar to the reduced activity seen during exposure to inhaled concentrations in the range of 10,000–15,000 ppm (6,20). The reduction in motoric effects is likely similar to the rate-decreasing effects that have been reported to occur with increasing doses of toluene, ethanol, and halothane in an operant behavioral task (13). Nevertheless, the combination of increased locomotor activity and the presence of obvious stereotypic behaviors including ataxia is similar to the profile of another abused drug phencyclidine (PCP).

Previous studies have provided a strong correlation between enhanced DA levels and increased locomotor activity following administration of several abused drugs (19). The foci of the DA-mediated hyperexcitability most likely lies within the mesolimbic DA system, whose fibers originate within the ventral tegmental area and project to the accumbens. Kelly et al. showed that 6-hydroxydopamine lesions of the accumbens blocked the characteristic hyperexcitability induced by amphetamine in rats (9). The argument that the hyperactivity detected following toluene administration may also be related to increased DA receptor activity is strengthened by the observation that pretreatment with the D_2 antagonist remoxipride blocked the characteristic hyperactivity seen with toluene. Mesolimbic dopamine systems are further implicated because remoxipride has been reported to preferentially bind to D_2 receptors in the nucleus accumbens rather than in the striatum (14). However, neither the exact site or mechanism by which toluene stimulates locomotor behavior has been delineated, nor to date have microdialysis measurements in the accumbens been carried out during toluene exposure. Nevertheless, we now have evidence that the electrophysiological response of midbrain dopamine neurons to toluene is qualitatively quite similar to the effects seen with

PCP and other noncompetitive *N*-methyl-D-aspartate antagonists (4).

Yet, Stengard et al. did perform microdialysis experiments in the striatum, a target structure for the A9 dopamine system during toluene administration (16). The striatum communicates with the substantia nigra by a DA–GABA loop, not unlike the DA–GABA loop existing between the VTA and nucleus accumbens. Toluene induced a 47% increase in extracellular striatal DA concentrations that were associated with a decrease in D_2 receptor affinity and were conjectured to result from a blockade of dopamine reuptake. Likewise, toluene was found in another study to decrease extracellular GABA in the globus pallidus through an increase in striatal DA activity (17). One mechanism by which this could occur could be through a toluene-induced stimulation of dopamine neurons in the substantia nigra.

Accordingly, toluene may increase DA levels within the NAcc by either directly stimulating A10 DA cell bodies (which project to the NAcc) or indirectly by inhibition of the GABA interneurons also residing within the ventral tegmental area. The latter would resemble the effects of the disinhibitory effects on A10 dopamine neurons by opiates (8). Both of the later hypotheses are consistent with data from our laboratory showing that inhalation of toluene vapor (11,000 ppm) can alter both dopamine and nondopamine neuronal firing in the ventral tegmentum (unpublished observations).

In conclusion, the present study demonstrates that biphasic alterations in spontaneous locomotor activity following intraperitoneal injections of toluene are likely coupled to increased DA function in mesolimbic structures. Further research will be needed to elucidate the mechanism for this effect of toluene.

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