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ORIGINAL INVESTIGATION

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Involvement of NO/cGMP pathway in toluene-induced locomotor hyperactivity in female rats

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Abstract *Rationale:* Nitric oxide (NO) is implicated in the acute locomotor activating effects of some addictive drugs such as amphetamine, caffeine, and PCP, but has not been investigated in the case of toluene. *Objectives:* This study determined the contribution of the NO-cyclic GMP (cGMP) pathway to locomotor stimulant effects of toluene. *Methods:* Locomotor activity was measured for 90 min immediately following toluene (500–1,000 mg/kg, IP) or corn oil treatments in Sprague-Dawley female rats. A NO generator, sodium nitroprusside (SNP) (3 and 6 mg/kg), a NO precursor, L-arginine (L-Arg) (250 mg/kg), a NO synthase inhibitor,N^G-nitro-L-arginine methyl ester (L-NAME) (5–20 mg/kg, IP), and a soluble guanylyl cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (10 mg/kg) were injected 5 min before toluene (750 mg/kg, IP) treatment. The combination effects of SNP with L-NAME, L-arginine with L-NAME, SNP with ODQ and L-arginine with ODQ on toluene-induced locomotor hyperactivity were also determined. *Results:* The locomotor hyperactivity induced by toluene was significantly inhibited by SNP and L-arginine, but enhanced by L-NAME and ODQ. SNP and L-arginine completely reversed the combined effects of L-NAME and toluene to a basal level and abolished the enhancing effects of ODQ. *Conclusions:* The results suggested that NO/cGMP-dependent mechanism might be involved in toluene-induced locomotor activity in rats.

Keywords Toluene · Nitric oxide · Cyclic GMP · Locomotor activity · Rat

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Introduction

Nitric oxide (NO) is an important intracellular messenger in the central nervous system and may also operate as a neurotransmitter. Release of NO occurs as a consequence of glutamate stimulation of *N*-methyl-D-aspartate (NMDA) receptors and is dependent upon calcium-calmodulin activation of the enzyme NO synthase (NOS). NO has been suggested to have multiple targets, among which the soluble guanylate cyclase (GC) is the most extensively characterized (Yun et al. 1997). Soluble GC converts guanosine 5′-triphosphate (GTP) to the important intracellular messenger cyclic guanosine 3′,5′-monophosphate (cGMP) (Denninger and Marletta 1999). NO has been also implicated in the development of dependence to substances such as morphine, ethanol, amphetamine, and nicotine (Tayfun Uzbay and Oglesby 2001). Recently, the role of NO has been studied in motor hyperactivity induced by a variety of drugs of abuse, including amphetamine, cocaine, phencyclidine (PCP), and caffeine. It has been reported that the NO donors can potentiate the hyperactivity induced by amphetamine, cocaine (Przegalinski and Filip 1997), and caffeine (Kayir and Uzbay 2003), but block the hyperactivity induced by PCP (Bujas-Bobanovic et al. 2000a). On the other hand, NOS inhibitors reverse the locomotor hyperactivity induced by amphetamine, cocaine (Celik et al. 1999), and caffeine (Kayir and Uzbay 2003), but potentiate the hyperactivity induced by PCP (Noda et al. 1995). Since the locomotor stimulatory properties of psychostimulants have been suggested to be related to their addictive potential (Wise and Bozarth 1987) and the inconsistent effects of NO in the locomotor activity induced by a variety of drugs of abuse, it is of interest to study the role of NO in the locomotor hyperactivity induced by other abused drugs.

Toluene is one of the most widely used industrial aromatic solvents. In addition to the industrial uses, toluene is abused via inhalation. This is done by “sniffing” (i.e. inhaling vapors from an open container), “bagging” (i.e. inhaling more concentrated vapors from a closed container), or “huffing” (i.e. breathing through a solvent-

soaked cloth) (Kurtzman et al. 2001). It is estimated that abusers may inhale from 4000 to 12,000 ppm toluene, taking multiple inhalations over a period of several minutes with repetitive dosing continuing for many hours (Bruckner and Peterson 1981a,b). Acute intoxication with toluene produces euphoria and disinhibition followed by hallucinations, tinnitus, ataxia, confusion, nausea and vomiting (Flanagan et al. 1990). Long-term abuse of toluene may induce marked brain atrophy together with dysfunctions of the CNS including cognitive impairment, ataxia, and paranoid psychosis (Goldbloom and Chouinard 1985; Byrne et al. 1991).

In animal experiments, toluene at low to intermediate doses can increase locomotor activity, with an observable increase in the frequency of sniffing and rearing and at high doses cause ataxia (Kjellstrand et al. 1985; Hinman 1987; Wood and Colotla 1990; Kondo et al. 1995; Riegel and French 1999; Wiaderna and Tomas 2000). The complex behavioral syndrome consisting of locomotor hyperactivity, stereotyped behavior, and ataxia is similar to the profile of PCP. PCP, an NMDA receptor antagonist, exerts its effects by binding within the ion channel of the NMDA receptor complex. Toluene is also reported to abolish NMDA-stimulated currents (Cruz et al. 1998). Thus, similar modulatory effects of NO on PCP-induced and toluene-induced locomotor hyperactivity are expected.

The present study was undertaken to investigate the contribution of NO/cGMP pathway in the toluene-induced locomotor hyperactivity. We used a NO generator, sodium nitroprusside (SNP), a NO precursor, L-arginine (L-Arg), and a NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) to determine the involvement of NO in the toluene-induced locomotor hyperactivity. In addition, the effects of 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, an inhibitor of soluble guanylyl cyclase) on toluene-induced locomotor hyperactivity were also tested.

Materials and methods

Animals

Adult gonadally intact female Sprague-Dawley rats (NSC Animal Center, Taiwan) weighing 200–250 g were used at random times in their oestrus cycle in this study. The oestrus cycle phases were determined by vaginal smear. The rats arrived at the animal facilities at least 5 days prior to the start of the experiments. Rats were housed in groups of three on a 12/12 light–dark cycle (lights on 0700 hours) at 22°C. Food and water were available ad libitum during the time the animals were in their home cages. All experiments were performed in accordance with the Republic of China animal protection law (Chapter III: Scientific Application of Animals) and approved by Review Committee of the Tzu Chi University.

Drugs

Toluene (HPLC grade 99.8%, Mallinckrodt Baker, Inc., Kentucky, USA) was administered in a corn oil. SNP,L-Arg, and L-NAME (Sigma) were dissolved in saline. ODQ (Sigma) was dissolved in dimethyl sulfoxide (DMSO) and was made up to final volume by addition of saline. DMSO content was 15%. All compounds were injected (1 ml/kg) intraperitoneally, but toluene (1000 mg/kg) was given as 2 ml/kg.

Experimental schedule

On experimental days, the animals were moved from the home cage, weighed and placed into activity cage (TruScan, Coulbourn Instruments, Pa., USA). They were allowed to habituate to the activity cage for 1 h. The first experiment was performed with various doses of toluene (500, 750, 900, 1000 mg/kg) without pretreatment. Each animal received a single treatment. The observation period started immediately after toluene or oil injection. Distance (cm) traveled per 10 min interval was recorded for 90 min. The dose of 750 mg/kg was chosen for the following experiments because it produced maximal locomotor response with no sign of motor coordination impairment and ataxia, which were assessed for 1 min at the end of every 10-min period after the injection by a well-trained observer blind to the treatment.

The doses of L-NAME (5–20 mg/kg), L-Arg (250–500 mg/kg), SNP (3 and 6 mg/kg), and ODQ (10 mg/kg) were chosen to be effective via systemic treatment on the basis of previous studies (Bujas-Bobanovic et al. 2000a,b; Heiberg et al. 2002). All the drugs were administered 5 min before toluene or oil injection. L-Arg and SNP were administered 5 min beforeL-NAME and/or ODQ injection when they were combined. The observation period started immediately after the last injection. Distance (cm) traveled per 10 min interval was recorded for 90 min. All experiments were carried out during the light phase (0900–1600 h)ours.

Statistical analysis

The total distances traveled during 90 min after injection of vehicle or toluene are presented as the mean±SEM. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey HSD test for post hoc comparisons. The level of significance was set at $P<0.05$.

Results

Intraperitoneal injection of toluene produced dose-related changes in locomotor activity. Toluene enhanced locomotor activity as compared with controls [$F(4,33)=2.99$, $P<0.05$] (Fig. 1). The higher doses of toluene, 900 and 1000 mg/kg, resulted in severe ataxic and lower locomotor

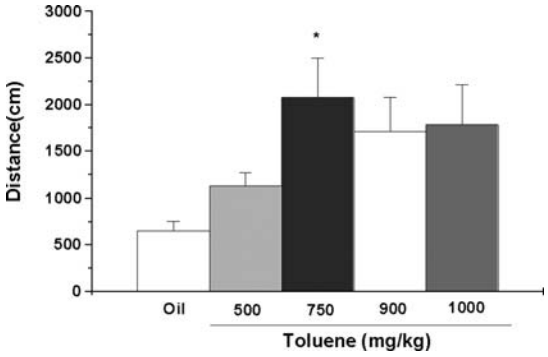

Fig. 1 Effects of toluene on locomotor activity as a function of dose. Rats treated with vehicle, and toluene of 500, 750, 900, and 1000 mg/kg. Data are expressed as mean±SEM distance traveled during 90 min after injection of vehicle or toluene (n=6–8). The *asterisk* indicates a significantly difference (P<0.05) from vehicle control

activity. Because of the robust locomotor effect and minimal interference from ataxia, 750 mg/kg toluene was chosen for further testing.

Treatment of SNP,L-Arg, L-NAME, and ODQ alone had no effect on the behaviors in rats. SNP (6 mg/kg) and L-Arg (250 mg/kg) pretreatment blocked the toluene-induced locomotor activity [F(6,42)=5.177, P<0.001] (Fig. 2). L-NAME (10 mg/kg) pretreatment enhanced the toluene-induced locomotor activity and SNP and L-Arg prevented the enhancing effects of L-NAME [F(7,42) =8.178, P<0.001] (Fig. 3). ODQ (10 mg/kg) pretreatment resulted in a significant enhancement of the toluene-induced locomotor activity. L-Arg (500 mg/kg) and SNP (6 mg/kg) reduced the enhancing effects of ODQ [F(6,38) =43.57, P<0.001] (Fig. 4).

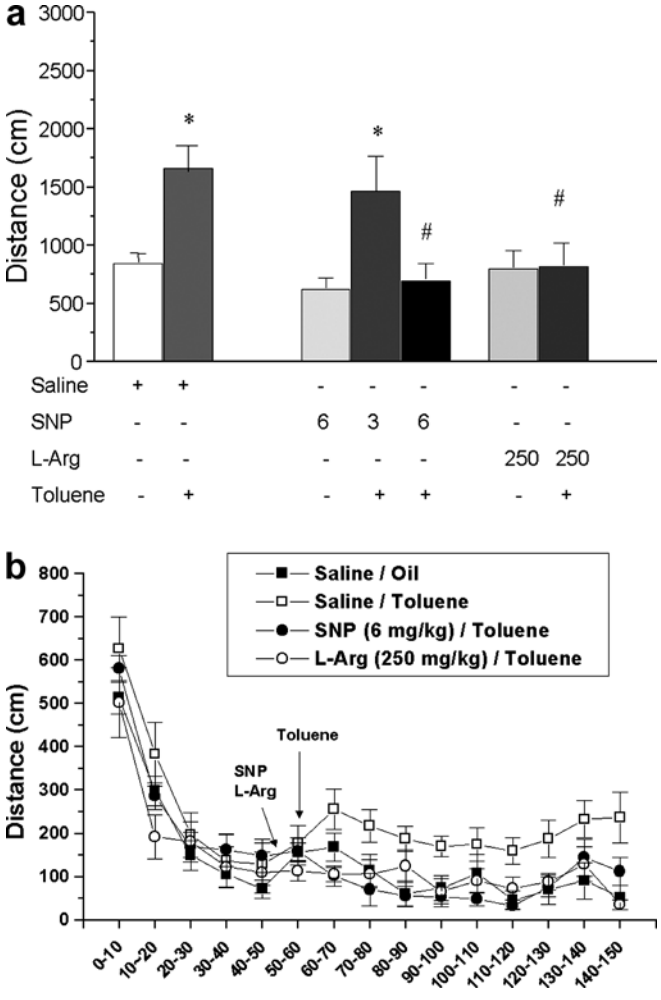

Fig. 2 Effects of SNP (3 and 6 mg/kg) and L-Arg (250 mg/kg) on toluene-stimulated locomotor activity. Data are expressed as mean ±SEM distance traveled **a** during 90 min after injection of vehicle or toluene (n=6–8), as well as **b** per 10-min interval for certain treatments. *Significantly different from vehicle+vehicle (P<0.05); #Significantly different from vehicle+toluene (P<0.05)

Discussion

The results show that toluene induced dose-dependent, biphasic alterations in locomotor activity and this effect was attenuated by SNP and L-Arg. On the other hand, L-NAME and ODQ enhanced the toluene-induced locomotor hyperactivity. The enhancing effects ofL-NAME and ODQ have been blocked by SNP and L-Arg. Overall, our findings indicated that there is a relationship between central NO/cGMP pathway and locomotor hyperactivity induced by toluene in rats.

The central mechanisms of action of toluene have been widely investigated. The results of these studies indicate a wide spectrum of interactions with various neuronal systems, such as glutamatergic, GABAergic, dopaminergic, serotonergic, and cholinergic systems (Balster 1998). NMDA receptor inhibition might be a mechanism that is directly related to toluene-induced behavioral effects because the neurobehavioral profiles of toluene overlap with those of NMDA receptor antagonists, such as PCP, MK801, and, ketamine, which produce similar behavioral effects consisting of locomotor hyperactivity, stereotyped sniffing, head movement and ataxia (Yang et al. 1991; Irifune et al. 1995). The effects of L-NAME and SNP on toluene-induced locomotor hyperactivity were similar to those seen previously with PCP (Noda et al. 1995; Bujas-Bobanovic et al. 2000a,b), indicating that the toluene-induced locomotor hyperactivity may also be mediated by blockade of NMDA receptors. However, the effects of L-Arg on locomotor hyperactivity stimulated by toluene or PCP are different. It has been demonstrated that L-Arg does not interfere with the behavioral effects induced by PCP alone, but reverses the enhancement of PCP-induced locomotor hyperactivity pretreated with L-NAME (Noda et al. 1995). However, that locomotor hyperactivity induced by toluene alone or L-NAME plus toluene is prevented by L-Arg. Based on the similar effects of SNP on toluene-induced and PCP-induced locomotor hyperactivity as well as administration ofL-Arg resulting in incremental elevations in brain NO in rats (Heinzen and Pollack 2003), it is possible that the animal species (mice versus rats) may account for the discrepant effects of L-Arg on PCP and toluene-induced locomotor hyperactivity.

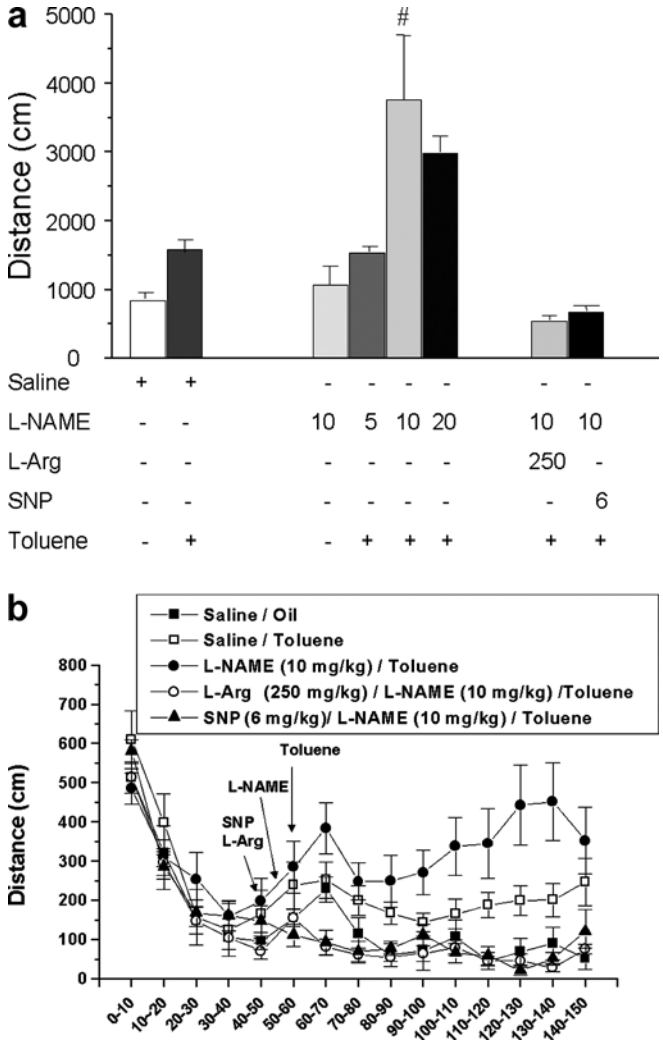

Fig. 3 Effects ofL-NAME (5, 10, and 20 mg/kg) and combination effects of SNP (6 mg/kg) with L-NAME (10 mg/kg) and L-arginine (250 mg/kg) with L-NAME (10 mg/kg) on toluene-stimulated locomotor activity. Data are expressed as mean±SEM distance traveled **a** during 90 min after injection of oil or toluene (n=6–8), as well as **b** per 10-min interval for certain treatments. #Significantly different from vehicle+toluene (P<0.05)

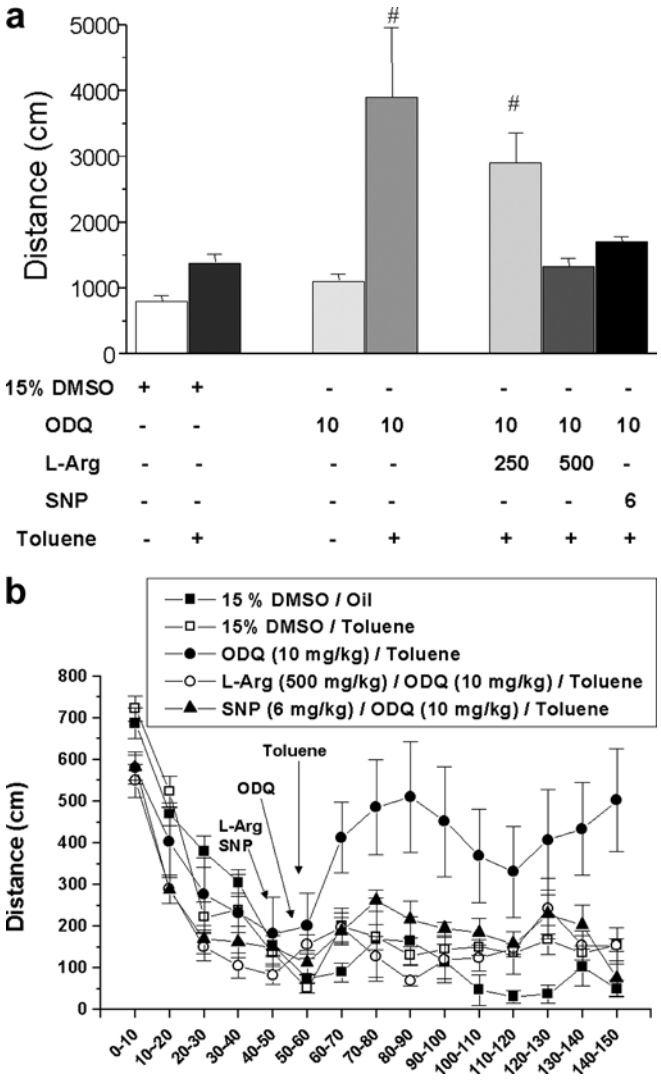

Fig. 4 Effects of ODQ (10 mg/kg) and combination effects of SNP (6 mg/kg) with ODQ (10 mg/kg) and L-arginine (250 and 500 mg/kg) with ODQ (10 mg/kg) on toluene-stimulated locomotor activity. Data are expressed as mean±SEM distance traveled **a** during 90 min after injection of oil or toluene (n=6–8), as well as **b** per 10 min interval for certain treatments. #Significantly different from vehicle +toluene (P<0.05)

The activation of NMDA receptors has been shown to induce NO synthesis, which then activates soluble GC and leads to the formation of cGMP in the brain (Szabo 1996). Because L-Arg and SNP antagonize the effects of toluene, it is possible that blockade of NMDA receptors by toluene might lead to a decrease in NO and cGMP levels to induce locomotor hyperactivity. It has been shown that NOS inhibitors have several effects similar to NMDA receptor antagonists, such as PCP and its analogue MK801. For example, hippocampal long-term potentiation is inhibited by NMDA receptor antagonists and NOS inhibitors (O'Dell et al. 1991). They also produce cataleptic effects (Jewett et al. 1996), attenuate sensitization to the locomotor-stimulating effect of cocaine (Pudiak and Bozarth 1993) and reduce ischemic injury (Adachi et al. 1998). However, there is also evidence that inhibition of NOS has different or opposite effects as NMDA receptor antagonist (Deutsch et al. 1996; Johnson et al. 2000; Zhu and Barr 2000). In the present study, we found that treatment of L-NAME and ODQ alone did not produce locomotor hyperactivity, but they potentiated the action of toluene. It is unlikely that toluene-induced locomotor hyperactivity via simply direct reduction of NO and cGMP. However, the opposite effects of SNP/L-Arg andL-NAME/ODQ confirm that the NO/cGMP pathway may act as an important modulator to modify the toluene-induced locomotor hyperactivity.

The mechanism by which NO might modify toluene-induced locomotor hyperactivity is still unclear. Recently, it has been reported that NO reduces the inhibitory effect of ethanol on NMDA receptors (Costa et al. 2003). Similar to ethanol, toluene also inhibits NMDA receptors. It is possible that the modulatory effects of NO on the

sensitivity of NMDA receptors may contribute to the inhibitory effects of L-Arg and SNP on toluene-induced locomotor hyperactivity. It is noteworthy that toluene modifies the activities of $GABA_A$ receptors, nicotinic receptors, and serotonin 5-HT_3 receptors besides NMDA receptors. With the complexity of toluene's sites of action, the alternative targets of NO may exist.

In conclusion, the results of the present study demonstrate that NO/cGMP pathway modulates the locomotor stimulatory effect of toluene in a manner similar to PCP, but different from amphetamine, cocaine, caffeine and ethanol. These findings suggest that toluene and PCP may activate neural circuits to stimulate locomotor activity distinct from amphetamine, cocaine, caffeine, and ethanol.

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