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Full Length Research Paper

The role of nitric oxide in nicotine reward: A place preference study in rats

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This study was aimed at evaluating the possible effects of nitrergic drugs (L-arginine and NG-nitro-L-arginine methyl ester (L-NAME)) on nicotine reward and dopamine-related behaviors (locomotion, sniffing, rearing, and compartment entering) in rats. The purpose was to determine whether modulation of these behaviors is involved in the possible effect of nitrergic drugs on nicotine-induced place conditioning in rats. Conditioning scores and dopamine-related behaviors were counted in all animals. Results showed that nicotine (1.5 mg/kg, intraperitoneally (i.p.)), but not L-arginine or L-NAME, induced conditioned place preference (CPP); however, nicotine reduced the locomotion and rearing and L-arginine reduced locomotion and sniffing, but L-NAME did not change these behaviors. L-arginine decreased the expression of nicotine-induced CPP and locomotion, rearing and sniffing. Although pretreatment with L-arginine abolished acquisition of nicotine place conditioning, it did not affect dopamine-related behaviors on the testing day. Additionally, although pre-exposure of rats to various doses of L-NAME did not attenuate expression of CPP, it inhibited acquisition of CPP produced by nicotine injection and significantly reduced some of dopamine-related behaviors both in acquisition and expression of nicotine-induced CPP. In conclusion, interaction of nitric oxide and nicotine in the CPP paradigm may be, at least in part, dependent on alteration of dopamine-related behaviors by nitric oxide.

Key words: Nicotine, nitric oxide, place conditioning, dopamine-related behaviors.

INTRODUCTION

The catastrophic effects of tobacco use on public health and the economy have been clearly documented in numerous studies (Hauswald 1989; Lakier, 1992; Ezzati et al., 2003; Peto 1996; Jha et al., 2006; Pasupathi et al., 2009). Among the many chemical compounds that tobacco and its smoke contain (Dome et al., 2010), nicotine is the most effective psychoactive component that confers tobacco's addictive properties (Wonnacott et al., 2005). In the brain, neuronal α-7 nicotinic acetylcholine receptors (nAchRs), which have high calcium permeability and extensive distribution in dopamine systems, are postulated to be the main targets of nicotine for reward induction (Pert et al., 1985; Gott and Clementi, 2004; Wonnacott et al., 2005; Gott et al., 2006; Araghavan et al., 2008). Among the dopamine systems of the brain, the reward system (mesolimbic dopamine system) is where the typical drugs of abuse, including nicotine, exert their addictive properties (Mansvelder and Mc Gehee, 2002; Wonnacott et al., 2005; Araghavan et al., 2008; Livingstone and Wonnacott, 2009).

Previous studies have clearly illustrated that there is substantial interaction of nicotine, dopamine, and nitric oxide (NO) in the brain, and especially in the reward system. NO is a gaseous hydrophobic molecule with high diffusion ability that can easily cross cell membranes (Schulman 1997). Production of NO in neurons is achieved by activation of neuronal nitric oxide synthase (nNOS) stimulated by elevation of calcium concentrations (Guix et al., 2005). Neural NOS is present in both origins and projections of mesolimbic and nigrostriatal neurons

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(Vincent and Kimura, 1992; Kharazia et al., 1994; Gracy and Pickel, 1998; French et al., 2005; Liu et al., 2008), and its coexistence with nicotinic receptors is another indicator of nicotine-NO interaction in these regions. NO can inhibit monoamine transporters; thus, it can enhance dopamine concentration in dopamine synapses (Pogun et al., 1994; Kiss et al., 1999, 2004).

On the other hand, the nitrergic system of rat brains can be modulated by dopamine systems and nicotine (Morris et al., 1997; Pogun et al.,2000; Wang and Lau, 2001). Nicotine can increase dopamine concentration in the striatum, nucleus accumbens and other targets of dopamine systems (Imperato et al., 1986; Di Chiara and Imperato, 1988; Mifsud et al., 1989; Balfour et al., 2000), and the mesolimbic dopamine system plays a critical role in nicotine addiction (Corrigall et al., 1994; Fu et al., 2000). NO mediates burst firing of dopaminergic neurons of the rat ventral tegmental area (VTA) induced by nicotine (Schilstorm et al., 2004). Furthermore, nicotine may indirectly increase NO concentration through stimulation of glutamatergic terminals and activation of high calcium permeability NMDA glutamate receptors on dopamine neurons in the VTA (Garthwaite et al., 1989; Schilstorm et al., 2004). Among behavioral studies, a considerable body of evidence indicates that NO is involved in various aspects of drug abuse, including dependence, reward properties, and behavioral sensitization. For example, inhibition of nitric oxide synthase reduces symptoms of nicotine, opiate and ethanol withdrawal in rodents (Adams and Cicero 1998; Jain et al., 2008).

Furthermore, involvement of NO in nicotine, morphine, amphetamine and cocaine-induced locomotor sensitization has been established by previous studies (Celik et al., 1999; Gupta and Pandhi, 2000; Shim et al., 2002; Zarrindasta et al., 2003). Moreover, previous studies have indicated the involvement of NO in reward properties of drugs of abuse, such as morphine, cocaine and nicotine (Gholami et al., 2002, 2003; Karami et al., 2002; Pudiak and Buzarth, 2002; Sahraei et al., 2004a).

Despite the previous experiments showing that NO is involved in modulation of nicotine reward effects in mice (Sahraei et al., 2004a), the role of alterations in dopamine-related behaviors by NO in this reward mechanism have not been evaluated. Thus, the aim of the current study was to determine whether NO interacts in the reward mechanism of nicotine with the conditioned place preference (CPP) paradigm in rats, and if so, to determine how much of this interaction is due to effects of NO on dopamine-related behaviors.

MATERIALS AND METHODS

Experimental animals

In this study, male Wistar rats (Pasture Institute, Tehran, Iran) weighing 240 - 300 g were used. The adaptation period for all animals after delivery to the laboratory was at least five days. Each group of five rats was housed in separated home cages in a room with a 12/12 h light-dark cycle and all experiments were carried out during the light phase. All animals had free access to sufficient food and water and were randomly placed in different experimental groups (N = 6). Every rat was used only once in the experiments and was experimentally naive at the beginning of the experiments. All experiments were conducted in accordance with standard ethical guidelines and approved by the local ethical committee (The Medical Committee on the Use and Care of Animals, Baqiyatallah (a.s.) University, 81/021, July 10, 2002).

Drugs

The drugs used in this study were nicotine base, NG-nitro-L-arginine methyl-ester (L-NAME) and L-arginine (Sigma-Aldrich, CA, USA). Different doses of all drugs were dissolved in 1 ml of saline (0.9%) as vehicle. All drugs were administered intraperitoneally (i.p.) in volumes of 1 ml/kg of animal body weight. Control groups received vehicle (saline) according to the experimental procedures.

Apparatus

The wooden apparatus consisted of two identical sizes, side-by-side, cubic compartments (30×45×45 cm) made from wood (Karami et al., 2002). The compartments were separated by a removable dividing wall, and according to the experimental procedures, the wall was removed whenever it was necessary for the animals to have free access to both compartments. The two parts of the apparatus were designed differently to provide distinct tactile and visual cues for the animals in each compartment. Specifically, one of the compartments had a smooth floor and the other had a corrugated base. In addition, the compartments were striped differently with black ribbons on the walls. The rats showed no significant preference for either compartment and therefore we used an unbiased conditioned place preference paradigm for all experiments.

Behavioral testing

Similar to a previous study (Shoaib et al., 1994), to increase positive reinforcement properties of nicotine, before induction of CPP with nicotine, all the animals received once-daily injections of nicotine (1 mg/kg, i.p.) for two consecutive days and were then returned to their home cage. After this priming phase, induction of place preference was carried out in three phases. It should be noted that animals conditioned with either L-arginine or L-NAME did not receive nicotine pretreatment.

To begin the experiments, the separating wall was first withdrawn, and then each animal was placed into the apparatus for 10 min and had free access to explore both compartments. The conditioning phase was conducted on three consecutive days (days 2 to 4) with two conditioning sessions on each day. In this phase, on day 2, animals received nicotine trials at 9:00 a.m. and were immediately confined to the drug-paired compartment for 45 min by the separating wall. Six hours later at 15:00 p.m., animals received saline trials and were immediately confined to the saline-paired environment for the same amount of time. On day 3, the order was reversed and animals received saline in the morning and nicotine in the afternoon conditioning sessions. On day 4, conditioning sessions were the same as on day 2. On day 5, the separating wall was removed and each animal was placed separately into the apparatus for 10 min and its behaviors were recorded by a digital camera for later offline analysis. Conditioning scores for each rat were calculated as the time spent in drug-paired compartment minus the time spent in the saline-paired compartment (in seconds).

Before experiments were conducted, the floor of the apparatus compartments was divided into four equal size squares. Locomotor activity was measured in terms of the number of times each rat crossed the lines and entered new squares during the testing session. The number of rearing in which the animal stood on its back feet (whenever animal's forepaws were against the apparatus walls) and maintained an erect posture in each compartment of the apparatus was also counted. Sniffing was counted using the upward sniffing (sniffing when the animal was in an upright position) method in both compartments of the apparatus during the testing session. Finally, the number of times each animal passed from one compartment of the apparatus to the other was counted for compartment entering.

Experimental procedures

Nicotine dose-response relationship in place conditioning

In this experiment, we examined the effects of different doses of nicotine in induction of CPP. Five groups of rats were treated with nicotine (0.1, 0.5, 1 and 1.5 mg/kg i.p.) or saline during the conditioning days. The control group received saline in both compartments of the apparatus during this phase.

L-arginine dose-response relationship in place conditioning

To test the effect of different doses of L-arginine on conditioned responses, four groups of rats received L-arginine (1, 5 and 10 mg/kg i.p.) or saline during the conditioning phase. The control group received only saline during this phase.

L-NAME dose-response relationship on the induction of CPP

Saline or L-NAME (5, 10, 20 mg/kg i.p.) was administered to four groups of animals during the conditioning sessions. The control group received only saline during this phase.

Effect of L-arginine on the expression of nicotine-induced CPP

Following induction of CPP with nicotine (1.5 mg/kg i.p.) during conditioning days four groups of rats were treated with different doses of L-arginine (1, 5 and 10 mg/kg i.p.) or saline five minutes prior to the testing session.

Effect of L-arginine on acquisition of nicotine- induced CPP

Five minutes prior to nicotine (1.5 mg/kg i.p.) administration, four groups of rats were treated with different doses of L-arginine (1, 5 and 10 mg/kg i.p.) or saline during the conditioning days. All rats were tested the day after the last session of the conditioning phase in a drug-free state.

Effect of L-NAME on the expression of nicotine-induced CPP

Four groups of rats that had been conditioned with nicotine (1.5 mg/kg i.p.) were treated with different doses of L-NAME (5, 10 and 20 mg/kg i.p.) or saline 30 min prior to the testing session.

Effect of L-NAME on the acquisition of nicotine-induced CPP

Four groups of rats were pretreated with L-NAME (5, 10 and 20 mg/kg i.p.) or saline 30 min prior to administration of an effective dose of nicotine (1.5 mg/kg i.p.) during conditioning sessions. All rats were tested the next day without additional injections in the testing phase.

Statistical analysis

Data were expressed as means ± standard error of mean (SEM). All data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Differences with $P < 0.05$ were considered significant.

RESULTS

Effect of nicotine dose-response on conditioned place preference paradigm

The effects of different doses of nicotine (0.1, 0.5, 1 and 1.5 mg/kg i.p.) in nicotine primed rats are shown in Figure 1. Injection of nicotine (1.5 mg/kg i.p.) induced a significant increase in drug-paired compartment time, compared to saline-paired compartment time [$F (4, 25) = 8.11$, $P < 0.001$] (Figure 1). However, intraperitoneal injection of saline (control group) in drug-paired compartments did not produce any preference or aversion in either compartment. Tukey's *post hoc* analysis showed that nicotine doses of 0.5 and 1.5 mg/kg produced CPP and the most effective dose of nicotine for induction of CPP was 1.5 mg/kg.

Effect of nicotine dose-response on dopamine-related behaviors

Nicotine doses of 0.1, 1 and 1.5 mg/kg i.p. decreased locomotor activity [$F (4, 25) = 4.89$, $P < 0.01$] and doses of 1 and 1.5 mg/kg i.p. reduced rearing [$F (4, 25) = 4.27$, $P < 0.01$], compared with the control (saline) group (Table 1). However, no significant differences were observed between nicotine- and saline-treated animals in respect to sniffing and compartment entering behaviors.

Effect of L-arginine on the place preference paradigm

The effects of different doses of L-arginine on naive animals are shown in Figure 1. Results demonstrated that injection of L-arginine (1, 5 and 10 mg/kg i.p.) did not induce a significant increase or decrease in place preference in drug-paired compartments compared to saline-paired compartments.

Effects of L-arginine on dopamine-related behaviors

L-Arginine in doses of 5 and10 mg/kg decreased locomotor activity [$F (3, 20) = 7.42$, $P < 0.01$] and in all doses (1, 5 and 10 mg/kg i.p.) decreased sniffing [$F (3, 20) = 23$, $P < 0.001$], compared with the control (saline)

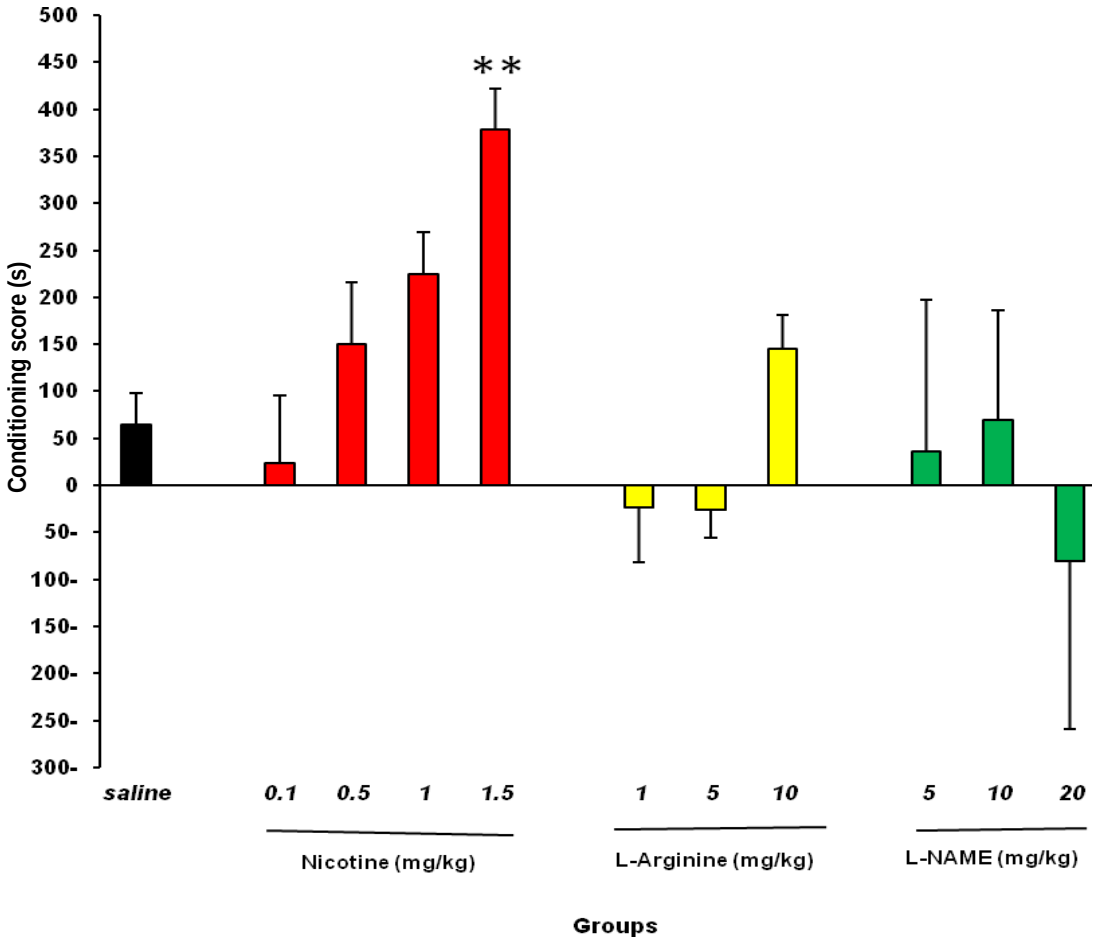

Figure 1. Conditioned place preference induced by nicotine, L-arginine and L-NAME. Each point represents the mean ± S.E.M. of conditioning scores for 6 to 8 rats. **P < 0.01compared with the saline control group.

group on the day of testing (Table 2); however, these treatments had no significant effect on rearing and compartment entering behavior.

Effect of L-NAME on the conditioned place preference paradigm

The effects of different doses of L-NAME (5, 10 and 20 mg/kg i.p.) on naive rats are shown in Figure 1. Results showed that injection of different doses of L-NAME did not induce any significant increase or decrease in time spent in the drug-paired compartment compared to the saline-paired compartment (Figure 1).

Effect of L-NAME on dopamine-related behaviors

Table 3 shows that treatment of rats with different doses of L-NAME (5, 10 and 20 mg/kg i.p.) on conditioning days had no significant effect on dopamine-related behaviors measured on the testing day relative to the control group.

Effect of L-arginine on expression of nicotine-induced CPP

Five minutes administration of different doses of L-arginine (1, 5 and 10 mg/kg i.p.) prior to the testing session in animals conditioned with effective doses of nicotine (1.5 mg/kg) decreased induction of place preference by nicotine (1.5 mg/kg) in a dose-dependent manner [$F(3, 20) = 5.16$, $P < 0.01$] (Figure 2).

Effect of L-arginine on expression of nicotine-induced dopamine-related behaviors

All dopamine-related behaviors other than compartment entering were significantly decreased by L-arginine injection in a dose-dependent manner (Table 4). Locomotor activity was reduced by an L-arginine dose of 1 mg/kg [$F(3, 20) = 3.64$, $P < 0.05$], rearing was reduced by L-arginine doses of 1, 5, and 10 mg/kg i.p. [$F(3, 20) = 7.69$, $P < 0.01$], and sniffing was attenuated by L-arginine doses of 1, 5, and 10 mg/kg i.p. [$F(3, 20) = 10.36$, $P < 0.001$].

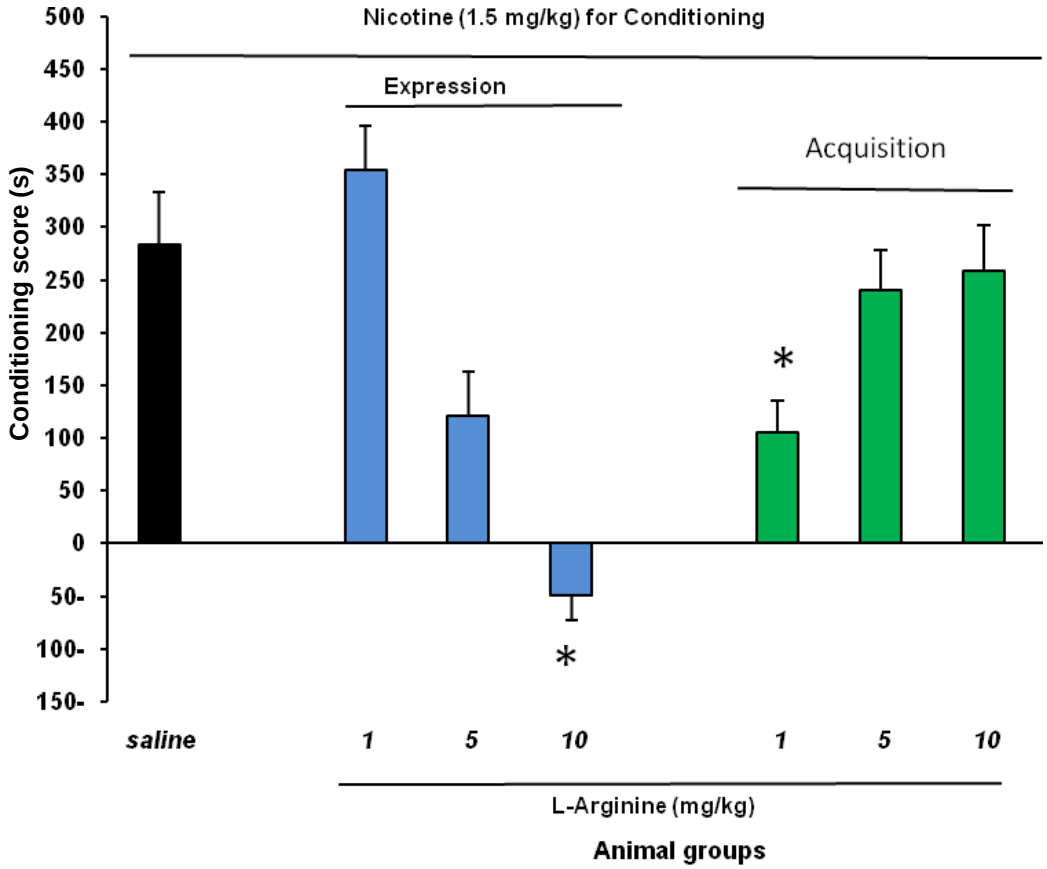

Figure 2. Effects of different doses of L-arginine (1, 5 and 10 mg/kg i.p.) on expression and acquisition of nicotine-induced CPP. Each point represents the mean ± SEM for 6 to 8 rats. *P < 0.05 compared with the saline control group.

Effect of L-NAME on expression of nicotine-induced CPP

Administration of different doses of L-NAME (5, 10 and 20 mg/kg i.p.) 30 min prior to the testing session had no significant effect on CPP induced by an effective dose of nicotine (Figure 3).

Effect of L-NAME on expression of nicotine-induced dopamine-related behaviors

L-NAME pretreatment 30 min before nicotine injection on the testing day decreased rearing [F (3, 20) = 6.52, P < 0.01] and sniffing [F (3, 20) = 5.65 P < 0.01] compared to the control group (Table 5).

Effect of L-arginine on acquisition of nicotine-induced CPP

Figure 3 shows that pretreatment with L-arginine (1 mg/kg) 5 min before injection of an effective dose of nicotine (1.5 mg/kg) on conditioning days abolished nicotine-induced CPP [F (3, 20) = 3.02, P < 0.05] compared to the control group .

Effect of L-arginine on acquisition of nicotine-induced dopamine-related behaviors

Pretreatment with L-arginine five minutes before injection of an effective dose of nicotine (1.5 mg/kg) on conditioning days did not affect any dopamine-related behaviors (Table 6).

Effect of L-NAME on acquisition of nicotine-induced CPP

Figure 3 shows that pretreatment with L-NAME (5 mg/kg i.p.) 30 min before injection of an effective dose of nicotine (1.5 mg/kg i.p.) significantly decreased acquisition of CPP [F (3, 20) = 7.27, P < 0.01] induced by the effective dose of nicotine compared to the control group.

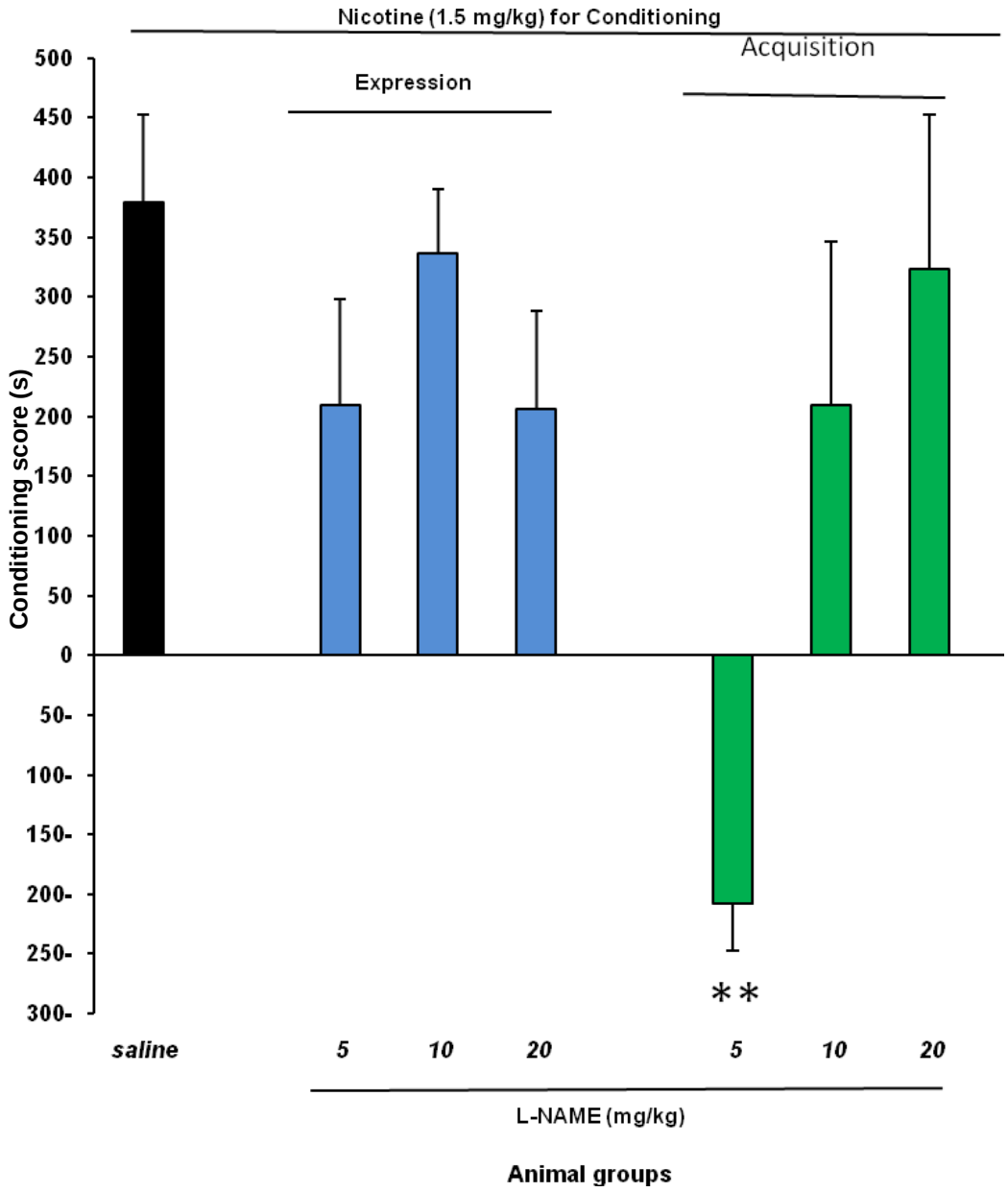

Figure 3. Effect of different doses of L-NAME (5, 10, and 20 mg/kg i.p.) on expression and acquisition of nicotine-induced CPP. Each point represents the mean ± SEM for 6 to 8 rats. **P < 0.01compared with the saline control group.

Table 1. Effect of different doses of nicotine on dopamine-related behaviors in rats.

Behavior / Groups	Locomotion (Counts/10 min)	Rearing (Counts/10 min)	Sniffing (Counts/10 min)	Compartment crossing (Counts/10 min)
Saline	100 ± 5.17	37 ± 1.43	19 ± 1.99	22 ± 1.83
Nicotine (0.1 mg/kg)	64 ± 3.37*	29 ± 2.18	24 ± 2.42	15 ± 1.21
Nicotine (0.5 mg/kg)	84 ± 7.15	29 ± 5.47	32 ± 5.7	18 ± 2.72
Nicotine (1 mg/kg)	68 ± 8.29*	23 ± 2.65*	17 ± 2.78	15 ± 2.38
Nicotine (1.5 mg/kg)	63 ± 10.13**	19 ± 3.14**	14 ± 2.49	13 ± 2.77

Each point represents the mean ± S.E.M. of locomotion, rearing, sniffing or compartment entering counts in 10 min for 6 to 8 rats. *P < 0.05 and **P < 0.01 compared with the saline control group.

Table 2. Dopamine-related behaviors induced by L-arginine in rats.

Behavior / Groups	Locomotion (Counts/10 min)	Rearing (Counts/10 min)	Sniffing (Counts/10 min)	Compartment crossing (Counts/10 min)
Saline	117 ± 7.77	46 ± 2.43	40 ± 1.42	20 ± 1.55
L-Arginine (1 mg/kg)	96 ± 6.21	37 ± 3.24	26 ± 1.89***	19 ± 1.25
L-Arginine (5 mg/kg)	72 ± 6.7**	33 ± 3.78	24 ± 2.38***	15 ± 1.15
L-Arginine (10 mg/kg)	67 ± 12.14**	31 ± 6.7	18 ± 1.76***	15 ± 2.85

Each point represents the mean ± S.E.M. of locomotion, rearing, sniffing or compartment entering counts in 10 min for 6 to 8 rats. **P < 0.01 and ***P < 0.001 compared with the saline control group.

Table 3. Dopamine-related behaviors induced by L-NAME in rats.

Behavior / Groups	Locomotion (Counts/10 min)	Rearing (Counts/10 min)	Sniffing (Counts/10 min)	Compartment crossing (Counts/10 min)
Saline	92 ± 6.46	38 ± 5.74	37 ± 4.86	17 ± 0.87
L-NAME (5 mg/kg)	72 ± 5.79	26 ± 2.16	25 ± 4.07	14 ± 1.92
L-NAME (10 mg/kg)	71 ± 8.53	19 ± 3.13	20 ± 3.63	18 ± 3.11
L-NAME (20 mg/kg)	81 ± 9.04	27 ± 7.74	30 ± 8.23	17 ± 1.60

Each point represents the mean ± S.E.M. of locomotion, rearing, sniffing or compartment entering counts in 10 min for 6 to 8 rats.

Table 4. Effect of different doses of L-arginine (1, 5 and 10 mg/kg i.p.) on expression of dopamine-related behaviors induced by nicotine in rats.

Behavior / Groups	Locomotion (Counts/10 min)	Rearing (Counts/10 min)	Sniffing (Counts/10 min)	Compartment crossing (Counts/10 min)
Saline	84 ± 7.88	36 ± 3.37	37 ± 3.6	13 ± 1.64
L-Arginine (1 mg/kg)	56 ± 5.78*	17 ± 2.72**	14 ± 1.85***	9 ± 1.53
L-Arginine (5 mg/kg)	59 ± 4.51	21 ± 1.47*	19 ± 1.97**	10 ± 1.02
L-Arginine (10 mg/kg)	76 ± 8.73	24 ± 3.88*	22 ± 4.13**	14 ± 2.40

Each point represents the mean ± S.E.M. of locomotion, rearing, sniffing or compartment entering counts in 10 min for 6 to 8 rats. *P < 0.05, **P < 0.01, ***P < 0.001 compared with the saline control group.

Table 5. Effect of different doses of L-NAME (5, 10 and 20 mg/kg i.p.) on expression of dopamine-related behaviors induced by nicotine in rats.

Behavior / Groups	Locomotion (Counts/10 min)	Rearing (Counts/10 min)	Sniffing (Counts/10 min)	Compartment crossing (Counts/10 min)
Saline	87 ± 10.94	36 ± 3.46	33 ± 3.41	13 ± 1.35
L-NAME (5 mg/kg)	96 ± 9.02	31 ± 4.11	29 ± 3.57	18 ± 2.44
L-NAME (10 mg/kg)	73 ± 12.41	19 ± 3.60*	17 ± 3.59*	14 ± 2.05
L-NAME (20 mg/kg)	72 ± 6.01	18 ± 2.74**	19 ± 2.78*	14 ± 1.45

Each point represents the mean ± S.E.M. of locomotion, rearing, sniffing or compartment entering counts in 10 min for 6 to 8 rats. *P < 0.05, **P < 0.01compared with the saline control group.

Effect of L-NAME on acquisition of nicotine-induced dopamine-related behaviors

L-NAME (10 mg/kg i.p.) pretreatment 30 min before nicotine (1.5 mg/kg) administration on conditioning days induced a significant decrease in locomotor activity [F (3, 20) = 2.80, P < 0.05], but it had no significant effect on other dopamine-related behaviors assessed on the

Table 6. Effects of different doses of L-arginine (1, 5 and 10 mg/kg; i.p.) on the acquisition of dopamine-related behaviors induced by nicotine in rats.

Behavior / Groups	Locomotion (Counts/10 min)	Rearing (Counts/10 min)	Sniffing (Counts/10 min)	Compartment crossing (Counts/10 min)
Saline	72 ± 7.79	22 ± 1.47	22 ± 1.88	15 ± 1. 4
L-Arginine (1 mg/kg)	65 ± 8.08	18 ± 3.69	18 ± 1.48	15 ± 2.53
L-Arginine (5 mg/kg)	66 ± 9.19	25 ± 5.90	24 ± 4.80	14 ± 1.87
L-Arginine (10 mg/kg)	62 ± 9.84	22 ± 3.53	21 ± 3.54	12 ± 2.76

Each point shows the mean±S.E.M of locomotion, rearing, sniffing or compartment entering counts in 10 min for 6 to 8 rats.

Table 7. Effects of different doses of L-NAME (5, 10 and 20 mg/kg; i.p.) on the acquisition of dopamine-related behaviors induced by nicotine in rats.

Behavior / Groups	Locomotion (Counts/10 min)	Rearing (Counts/10 min)	Sniffing (Counts/10 min)	Compartment crossing (Counts/10 min)
Saline	72 ± 7.79	22 ± 1.47	22 ± 1.88	15 ± 1.4
L-NAME (5 mg/kg)	59 ± 5.91	20 ± 5.63	19 ± 5.45	11 ± 0.8
L-NAME (10 mg/kg)	43 ± 2.11*	21 ± 3.23	18 ± 2.26	10 ± 1.43
L-NAME (20 mg/kg)	57 ± 10.09	22 ± 5.43	20 ± 5.59	13 ± 2.76

Each point shows the mean ± S.E.M. of locomotion, rearing, sniffing or compartment entering counts in 10 min for 6 to 8 rats, *P<0.05 compared with the saline control group.

testing day (Table 7).

DISCUSSION

Our results showed that nicotine-induced CPP, which is indicative of nicotine's hedonic effects in rats, was inhibited by nitrergic drugs, namely L-arginine and L-NAME. This inhibition is mediated, at least in part, through the influence of these drugs on components of the dopamine system. The evidence for this conclusion is that dopamine-related behaviors (sniffing, rearing, locomotor activity, and compartment entering) were affected by nitrergic drugs in animals conditioned with nicotine. Consistent with a number of earlier studies, the present investigation showed that nicotine induced a dose-dependent CPP in animals primed with nicotine and thus had prior exposure to the drug. Anatomically, nAChRs, which are located in various parts of the mesolimbic dopamine system including the VTA and nucleus accumbens (Clarke et al.,1985), play a critical role in the reward effects of nicotine and induction of CPP.

In contrast to our study, some studies demonstrated that in the absence of a priming regimen, nicotine conditioned a place preference in a dose-independent fashion in rats and mice (for rev see: Tzschentke, 2007). Thus, it is reasonable to conclude that a nicotine priming regimen facilitates development of reward pathways in the brain in such a way that administration of different doses of nicotine can produce a dose-dependent CPP. Numerous studies of the mechanisms of nicotine-induced CPP have shown that interference with dopamine, glutamate, cholinergic, and nitrergic systems can inhibit the reward effects of nicotine in rats and mice (for rev see: Tzschentke, 2007). For example, a previous study on mice showed that systemic administration of L-arginine inhibited nicotine-induced CPP and pretreatment with mecamylamine, a nicotinic acetylcholine receptor blocker, blocked induction of CPP by L-arginine (Sahraei et al., 2004b). In our study, dopamine-related behaviors changed significantly in animals conditioned with nicotine. These changes are another indication that the mesolimbic dopamine pathway is involved in mediation of nicotine reward. It is important to note that in our experiments animals were tested 24 h after the last nicotine conditioning session; therefore, modulations of dopamine-related behaviors after this time indicate that nicotine, in addition to directly activating the mesolimbic system, established new memories in the system that persisted for at least 24 h after nicotine injection.

Previous studies of induction of place preference by L-arginine have, however, reported contradictory results (Gholami et al., 2002; Sahraei et al., 2004a). Our results showed that L-arginine administration did not induce a significant CPP in rats. The discrepancies may be due to methodological differences because we used a two-part apparatus, while the other studies used a three-part apparatus. Moreover, our results are similar to those of Karami and colleagues, who did not observe a place

preference induced by L-arginine in a two-part apparatus (Karami et al., 2002). Regarding the ability of NO to inhibit monoamine transporters in the nucleus accumbens (Pogun et al., 1994; Kiss et al., 1999), it had been speculated that an increase in dopamine concentration caused by elevation of NO levels was responsible for L-arginine-induced CPP in some experiments; however, this mechanism is not sufficiently strong to induce CPP in all procedures. On the other hand, similar to previous studies, we found that L-NAME did not produce any place preference. However, because L-arginine could reduce dopamine-related behaviors on the testing day, the effect of L-arginine on the mesolimbic dopamine system might be compared with the effect of L-NAME.

Our results showed that administration of L-arginine inhibited acquisition of nicotine-induced CPP in rats. This effect is interesting because dopamine-related behaviors, including locomotor activity, rearing, sniffing, and compartment entering were not significantly different compared to the control group. Since it is well established that NO plays a critical role in long-term potentiation (LTP), it can be deduced that the reduction in acquisition of CPP mediated by L-arginine is caused by the effect of this drug on memory establishment processes involved in the reward effects of nicotine. In addition, there are many data that indicate nicotine can improve memory formation (Wonnacott et al., 2005). The first parts of our experiments in which L-arginine was shown to alter significantly dopamine-related behaviors have led us to suggest that L-arginine may affect both pathways involved in nicotine reward and other pathways involved in dopamine-related behaviors, such as the nigrostriatal system. However, L-arginine effects on the reward system may mask its effects on other systems. Similar to previous studies in mice, we found that L-NAME inhibited nicotine-induced place conditioning in rats. Furthermore, L-NAME treatment reduced locomotor activity on the testing day. Thus, it can be concluded that the observed reduction in conditioning scores on the testing day may be due to effects of L-NAME on the locomotor system of the brain, and not simply its effect on the reward properties of nicotine.

In our experiments, we also found that pretest administration of L-arginine but not L-NAME, reduced expression of nicotine place conditioning in rats, which was in agreement with previous studies in mice (Sahraei et al., 2004a). There is convincing evidence that this reduction does not directly result from the effects of the drug on nicotine function. For example, dopamine-related behaviors were attenuated after pretest administration of L-arginine, suggesting that the effect of L-arginine on the expression of nicotine CPP may involve interaction with locomotor and other behaviors and these interactions might lead to false responses. It should be noted that earlier studies indicated that L-arginine and nAChRs interacted directly, and inhibition of nAChRs abrogated L-arginine place conditioning (Sahraei et al., 2004a). Therefore, given the diversity of nAChR subtypes and their widespread distribution in the brain, the results of the current study may demonstrate direct or indirect effects of L-arginine on nAChRs somewhere other than within the mesolimbic dopamine system. On the other hand, L-arginine metabolism in the brain produces an extensive spectrum of intermediary compounds and each compound may have its own specific effects on the brain and the reward effects of nicotine (for rev see: Guix et al., 2005).

Moreover, it should be noted that in previous studies, dopamine-related behaviors have never been assessed during evaluation of expression or acquisition of the CPP paradigm, and therefore, the role of other parts of the brain dopamine system, such as nigrostriatal system, in acquired responses by the CPP paradigm had not been evaluated. Our study suggests that when using a CPP paradigm to assess reward properties of a drug, it is also necessary to evaluate dopamine-related behaviors because these behaviors are indicators of activity of different dopamine systems, to determine what portion of the observed drug effects are attributable to mesolimbic system activation by the drug. Nicotine in particular is similar to many drugs of abuse and the mesolimbic system also stimulates the nigrostriatal system. Therefore, evaluating dopamine-related behaviors as a criterion of activity in the mesolimbic and nigrostriatal systems is a good way of assessing the relative role of these systems in drug reinforcement.

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