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Ascorbic acid antagonizes nicotine-induced place preference and behavioral sensitization in mice

Hedayat Sahraei [a,*], Ali Akbar Aliabadi [b], Mohammad-Reza Zarrindast [c], Hassan Ghoshooni [a,d], Ali Nasiri [a], Amir Abbas Barzegari-Sorkheh [a], Maryam Yari [b], Homeira Zardooz [e], Leila Hossein-Mardi [b], Nasrin Faraji [b], Jamal Shams [e]

[a] Department of Physiology and Biophysics and Behavioral Sciences Research Center (BSRC), Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran
[b] Department of Biology, Azad University, North Branch of Tehran, Iran
[c] Department of Pharmacology, Tehran University of Medical Sciences, Iran
[d] Chemical Injuries Research Center, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran
[e] Neuroscience Research Center (NRC), Shaheed Beheshti University of Medical Sciences, Tehran, Iran

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Abstract

In the present study, the influence of ascorbic acid on the nicotine-induced behavioral sensitization and conditioned place preference was investigated in mice. In the place preference paradigm, intraperitoneal (i.p.) nicotine (1and 1.5 mg/kg, three drug sessions) but not ascorbic acid (1, 10, 100 and 1000 mg/kg) administration induced place preference. Ascorbic acid administration (10, 100 and 1000 mg/kg, i.p.) reduced both the acquisition and expression of nicotine-induced place conditioning. Locomotor sensitization in mice was produced by intraperitoneal injection of nicotine (0.25 mg/kg) for 7 consecutive days. On the 9th day of the experiments, activity of the mice was recorded after challenge with nicotine (0.1 mg/kg, i.p.). Ascorbic acid (10, 100 and 1000 mg/kg, i.p.) was injected 20 min before each injection of nicotine (acquisition of sensitization) or acutely 20 min before a challenge nicotine injection (expression of sensitization). It was shown that ascorbic acid attenuated the acquisition of nicotine sensitization in a dose-independent manner but the expression of nicotine-induced sensitization was not affected by ascorbic acid. In conclusion, it seems that ascorbic acid may interfere with nicotine-induced place preference and behavioral sensitization in mice.
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Keywords: Nicotine; Ascorbic acid; Place preference; Behavioral sensitization; (Mice)

1. Introduction

Chronic tobacco use has become a major health problem worldwide. Nicotine dependence has been accepted as a major cause underlying tobacco addiction (Baker et al., 2004).

The dopaminergic projections originating from the ventral tegmental area and projecting to the nucleus accumbens are considered as the main biological substrate of the reinforcing and stimulant effects of nicotine (Wonnacott et al., 2005; Baker et al., 2004; Picciotto, 2003; Di Chiara, 2000; Balfour et al., 1998). Several data confirmed that nicotine elevates the extracellular concentration of dopamine in the nucleus accumbens, in particular its shell subdivision (Cadoni and Di Chiara, 2000). However, recent studies have demonstrated an important role for the glutamate system within the ventral tegmental area in nicotine-induced dopamine release (Fu et al., 2000). Furthermore, repeated administration of the animals with nicotine is well known to cause a long-lasting increase in its psychomotor effects (Cadoni and Di Chiara, 2000; Booze et al., 1999). This process in behavioral sensitization has been suggested to play a role in the nicotine addictive behavior (Picciotto, 2003). The dopaminergic and glutamatergic innervations within the ventral tegmental area and nucleus accumbens have been implicated for nicotine sensitization as well (Picciotto, 2003).

Ascorbic acid is a water-soluble vitamin with antioxidant properties that accumulates in the brain at relatively high concentrations (Rice, 2000). Several investigations indicated

* Corresponding author. Department of Physiology and Biophysics, Baqiyatallah (a.s.) University of Medical Sciences, P.O. Box: 19395-6558, Tehran, Iran. Tel./fax: +98 21 22281561.
E-mail address: h.Sahraei@bmsu.ac.ir (H. Sahraei).

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that local applications of ascorbic acid enhanced the response of neurons to dopamine (Heikkila et al., 1981; Debler et al., 1988; Tolbert et al., 1992; Rebec and Pierce, 1994) and glutamate (Rice, 2000). In particular, the relationship between dopamine system and ascorbic acid are an interesting issue. For example, the vitamin acts as a dopamine antagonist in brain (Tolbert et al., 1992). Ascorbic acid inhibits the binding of radio labeled dopamine agonists and antagonists in the brain tissue (Tolbert et al., 1992). Ascorbic acid also attenuates the metamphetamine-induced dopamine release within the nucleus accumbens (Pierce et al., 1995). In addition, several reports indicated that ascorbic acid reduces behavioral changes induced by the dopamine agonist, D-amphetamine (Pierce et al., 1995).

Ascorbic acid interacts with nicotine dependence in human as well as in animal models. In this regard, it has been shown that ascorbic acid could reduce nicotine-induced nitric oxide release in the kidney in healthy voluntaries subjects (Halimi and Mimran, 2000). In addition, ascorbic acid supplementation reduces cigarette smoking (Dawson et al., 1999). Investigations also show that ascorbic acid aerosol can reduce the negative moods associated with cigarette cessation and cigarette craving (Levin et al., 1993). Nicotine can also reduce ascorbic acid plasma level both in men and women (Kim et al., 2004).

However, despite investigations regarding the effects of ascorbic acid on alcohol and amphetamine reward (Miquel et al., 1999; Pierce et al., 1995), little is known about the effects of ascorbic acid on nicotine reward properties. The main aim of this study is to provide further clarification for the role of ascorbic acid in nicotine positive reinforcement and sensitization in mice, which are among the important reward properties of the drug. For this purpose, we use the un-biased conditioned place preference paradigm as a model for investigation of nicotine reinforcing properties. In addition, locomotor activity was evaluated by mean of an activometer for behavioral sensitization.

2. Methods and materials

2.1. Animals

Female NMRI mice (20–25 g) were used ($n=7$–9/group). The animals were housed ten per cage in an animal room that was lit for 12 h per day (light on at 7:00 a.m.) in a temperature-controlled environment (23±1 °C). Food and water were available continuously. Each animal was used only once and the attention was paid to the ethical guidelines for investigations of experimental pain in conscious animals. All experiments were conducted in accordance with standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah (a.s.) University of Medical Committee on the Use and Care of Animals, 83/152, Jan 12, 2003).

2.2. Apparatus

The place preference apparatus based on the design of (Zarrindast et al., 2003) with modifications, was made of wood and consisted of two square-base compartments (15×15×15 cm). One compartment was painted in white color with black stripes (1 cm wide) and the other in black color with white stripes (1 cm wide). There was a texture in the ground area of the black compartment. Compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling.

Locomotor activity of the mice was measured in a rectangular activometer cage (30×30×30 cm, three light beams in the walls) kept in a sound-attenuated room.

2.3. Place preference paradigm

The conditioned place preference paradigm took place on 5 consecutive days by using an un-biased procedure (Animals' preference for the black compartment was 311±45 s and for white side was 295±56 s). In addition, the drug and control compartments were randomly assigned for each animal in a counterbalanced way.

2.3.1. Pre-conditioning

On day 1, each mouse was placed separately into the apparatus for 10 min, with free access to both compartments.

2.3.2. Conditioning

This phase consisted of a 3-day schedule of double conditioning sessions (i.e. days 2–4). The first day involved a morning session (9:00–11:00 h) in which the animals received a single intraperitoneal (i.p.) dose of nicotine and were placed immediately in one compartment for 30 min. This compartment had been isolated from the other using a removable partition. In the evening session (15:00–17:00 h) the animals received a single injection of saline, and were placed for 30 min in the other compartment. On the second day of conditioning the animals received the saline injections in the morning session and the drug administration in the evening session. The third day of conditioning had the same schedule as the first one.

2.3.3. Post-conditioning

On the day 5 of the schedule, as in the pre-conditioning phase, the partition was raised and the mice were placed in the apparatus (between white and black compartments) and allowed again to freely explore the two compartments and the time spent in the white or black compartment was recorded for 10 min for each mouse.

2.4. Locomotor activity

Locomotor activity was measured by means of three infrared LEDs attached to the wall of a rectangular activometer cage (30×30×30 cm). Animals were placed in the apparatus for 10 min for adaptation. After this time, each mouse received a single injection of different doses of nicotine, ascorbic acid or saline (as control) and replaced in the apparatus. Five minutes later, locomotor activity of the animals was evaluated for a 20 min period.

2.5. Development of nicotine sensitization

Sensitization to nicotine was achieved with the method based on previous work (Biala, 2003) with modifications. The mice

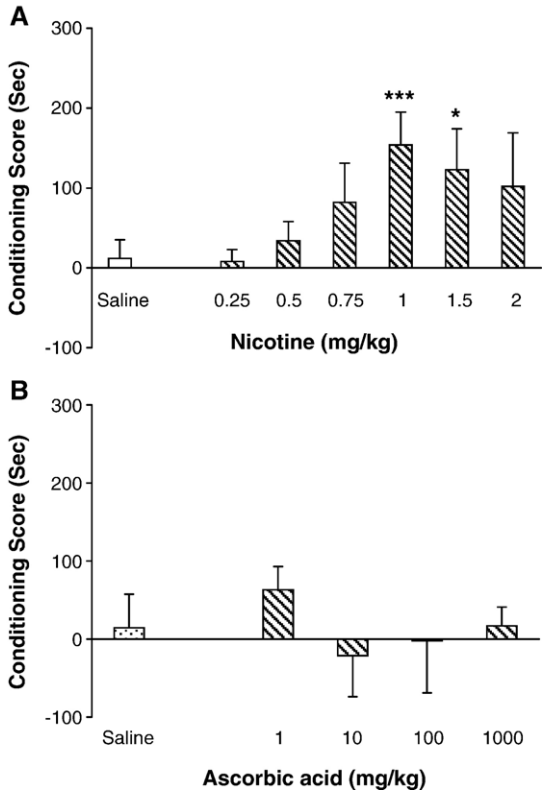

Fig. 1. The effects of nicotine (A) and ascorbic acid (B) on conditioned place preference paradigm. Animals received either saline (10 ml/kg) or different doses of nicotine (0.25, 0.5, 0.75, 1, 1.5 and 2 mg/kg) and/or ascorbic acid (1, 10, 100 and 1000 mg/kg) during a 3-day schedule of conditioning. Conditioning scores are defined as the time spent in drug-paired compartment minus that spent in the saline-paired compartment. Each point is the mean±S.E.M. for 7–9 mice. *$P<0.05$, ***$P<0.001$ difference from saline control group.

were treated intraperitoneally with nicotine (0.25 mg/kg) or saline (10 ml/kg) once daily (8:00 a.m.), for a period of seven days in the colony room. After one day of withdrawal (day 9), all animals were given a challenge dose of nicotine (0.1 mg/kg) to induce behavioral sensitization.

2.6. Drugs

The following drugs were used: nicotine base and ascorbic acid (Sigma, UK). The control groups received saline. All drugs were administered intraperitoneally (i.p.) in a volume of 10 ml/kg. Nicotine and ascorbic acid solutions were prepared in saline with the pH adjusted to 7.2±0.1.

2.7. Statistical analysis

One-way or Two-way analysis of variance (ANOVA), followed by Tukey test was used to evaluate the significance of the drugs. A value of $P<0.05$ was considered significant. For the Place preference paradigm, conditioning scores represent the time spent in the drug-paired compartment minus the time spent in the saline-paired place, and is expressed as the mean±S.E.M. Locomotor activity was expressed as a number of photocell beam breaks (mean±S.E.M.).

3. Results

3.1. Effects of nicotine and ascorbic acid on place conditioning paradigm

Administration of nicotine (0.25, 0.5, 0.75, 1, 1.5 and 2 mg/kg, i.p.) but not saline (10 ml/kg; i.p.) to the animals caused a significant increase in the time spent in the drug-paired compartment (i.e. place conditioning) [$F(6,50)=3.24$, $P<0.02$]. Post hoc analysis showed that two doses of nicotine (1 and 1.5 mg/kg) induced place conditioning (Fig. 1A). However, intraperitoneal injections of different doses of ascorbic acid (1, 10, 100 and 1000 mg/kg; i.p.) did not change the animals' tendency for the drug-paired compartment. [$F(4,45)=0.5$, $P>0.05$], (Fig. 1B).

3.2. Effects of ascorbic acid on the acquisition and expression of nicotine-induced conditioned place preference

When ascorbic acid (1–1000 mg/kg, i.p.) was administered 20 min before testing on the 5th day of experiments the expression of nicotine-induced conditioned place preference was reversed towards place aversion [two-way ANOVA; within-group comparison: ascorbic acid effect: $F(4,37)=6.23$, $P<0.001$, nicotine effect: $F(1, 36)=4.53$, $P<0.001$, ascorbic acid X nicotine: $F(4,37)=7.43$, $P<0.0001$] (Fig. 2). Furthermore, administration of ascorbic acid (1, 10, 100 and 1000 mg/kg, i.p.) 20 min before nicotine (1 mg/kg, i.p.) injection during the conditioning phase, also showed aversion on the test sessions [two-way ANOVA; within-group comparison: ascorbic acid effect: $F(4,39)=5.34$, $P<0.001$, nicotine effect: $F(1, 38)=6.30$, $P<0.001$, ascorbic acid X nicotine: $F(4,39)=8.12$, $P<0.0001$] (Fig. 2).

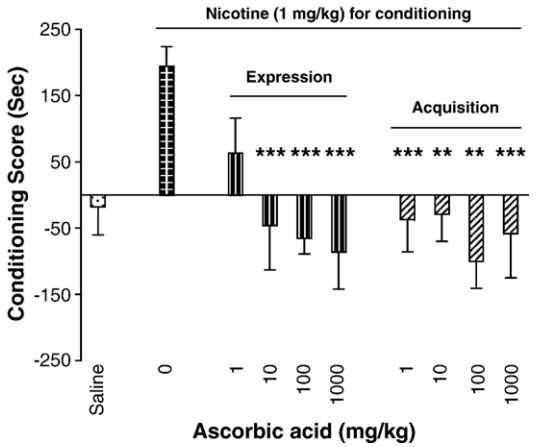

Fig. 2. Effects of ascorbic acid on the expression and acquisition of place preference induced by nicotine. One group of animals received a dose of nicotine (1 mg/kg) during a 3-day schedule of conditioning. The effects of different doses of ascorbic acid (1, 10, 100 and 1000 mg/kg) on the expression of nicotine-induced conditioning were tested on day 5 (test day). Another group of animals received a dose of nicotine (1 mg/kg) during a 3-day schedule of conditioning, in the presence of saline (10 ml/kg) or different doses of ascorbic acid (1, 10, 100 and 1000 mg/kg). Conditioning score is defined as in Fig. 1.A. Each point is the mean±S.E.M. for 7–9 mice. ***$P<0.001$ difference from control group.

3.3. Effects of nicotine and ascorbic acid on the animals' locomotor activity

In this series of the experiments for the evaluation of the effects of nicotine and ascorbic acid on the animals locomotion, different doses of nicotine (0.25, 0.5, 0.75, 1 and 1.5 mg/kg, i.p.) or ascorbic acid (1, 10, 100 and 1000 mg/kg, i.p.) were injected to the animals and 5 min later, the animals' activity was recorded during a 20 min period. Results showed that the activity was significantly reduced after nicotine administration, but the results are significant only for the dose 1 mg/kg of the drug [$F(5, 45)=3.42$, $P<0.01$] (Fig. 3A). However, administration of ascorbic acid did not change the animals' activity [$F(4, 25)=0.8$, $P>0.05$] (Fig. 3B).

3.4. Nicotine dose-response on behavioral sensitization

Mice were treated intraperitoneally with nicotine (0.25 mg/kg) or saline (10 ml/kg) once daily (8:00 a.m.), for a period of seven days. After one day of withdrawal (day 9), all animals were given a challenge dose of nicotine (0.05, 0.1 and 0.25 mg/kg) to induce behavioral sensitization. Results showed that nicotine sensitization could be better demonstrated at a dose of 0.1 mg/kg of nicotine [$F(3, 25)=3.21$, $P<0.001$] (Fig. 4).

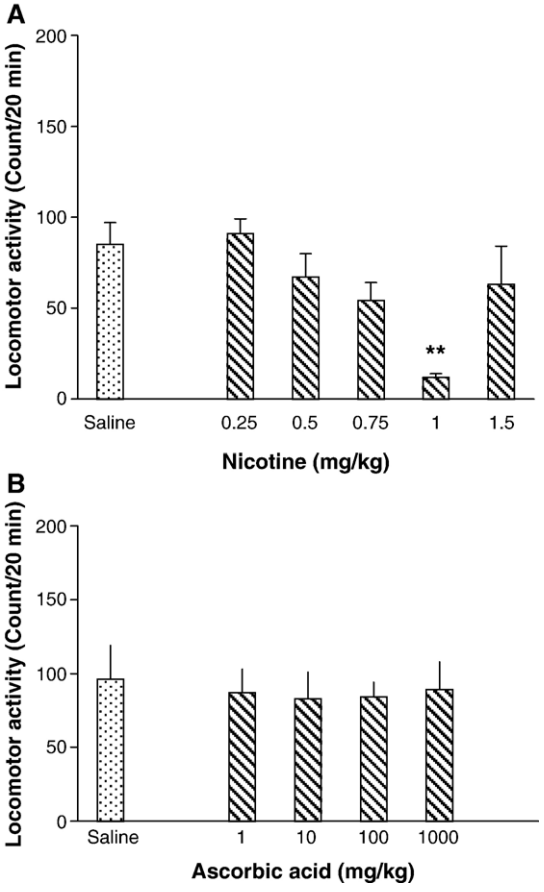

Fig. 3. A: Effects of nicotine on the animals' locomotor activity. In a group of animals, different doses of nicotine (0.25, 0.5, 0.75, 1 and 1.5 mg/kg, i.p.) were injected and 5 min later, the animals' activity was recorded during a 20 min period. The animals' activity was recorded as the number of photocell beam breaks. Each point is the mean±S.E.M. for 7–9 mice. $^{**}P<0.01$ difference from control group. B: Effects of ascorbic acid on the animals' locomotor activity. A group of animals received ascorbic acid (1, 10, 100 and 1000 mg/kg, i.p.) and 5 min later; the animals' activity was recorded during a 20 min period. Each point is the mean±S.E.M. for 7–9 mice.

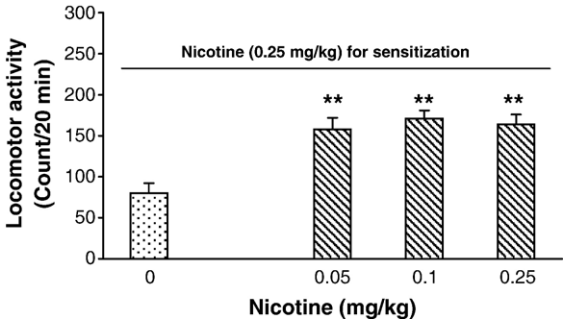

Fig. 4. Nicotine dose-response on behavioral sensitization. Mice were injected intraperitoneally with nicotine (0.25 mg/kg) or saline (10 ml/kg) once daily, for a period of seven days. After one day of withdrawal (day 9), all animals were given a challenge dose of nicotine (0.05, 0.1 and 0.25 mg/kg) to induce behavioral sensitization. The animals' activity was recorded as the number of photocell beam breaks. Each point is the mean±S.E.M. for 7–9 mice. $^{**}P<0.001$ difference from control group.

3.5. Effects of ascorbic acid on the acquisition and expression of nicotine-induced behavioral sensitization

Animals received an injection of nicotine (0.25 mg/kg, i.p.) once daily for 7 days in the colony room. Locomotor activity was measured one day after the termination of injections on day 9. Ascorbic acid (1, 10, 100 and 1000 mg/kg, i.p.) or saline (10 ml/kg) were administered to the animals 20 min before each nicotine injection during the development of sensitization. Fig. 5 indicated that repeated administration of nicotine sensitized the animals [two-way ANOVA; within-group comparison:

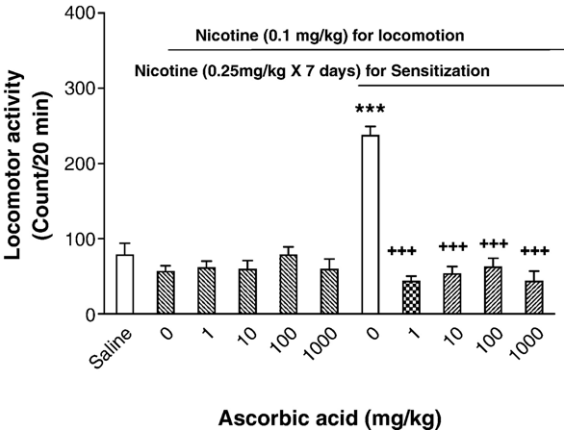

Fig. 5. Effects of ascorbic acid on the acquisition of nicotine-induced behavioral sensitization. The animals received an injection of nicotine (0.25 mg/kg, i.p.) once daily for 7 days in the colony room. Locomotor activity commenced one day after the termination of injections on day 9. Ascorbic acid (1, 10, 100 and 1000 mg/kg, i.p.) or saline (10 ml/kg) was administered to the animals 20 min before each nicotine injection during the development of sensitization. The animals' activity was recorded as the number of photocell beam breaks. Each point is the mean±S.E.M. for 7–9 mice. $^{***}P<0.001$ compared with saline control group. $^{+++}P<0.001$ compared with nicotine control group.

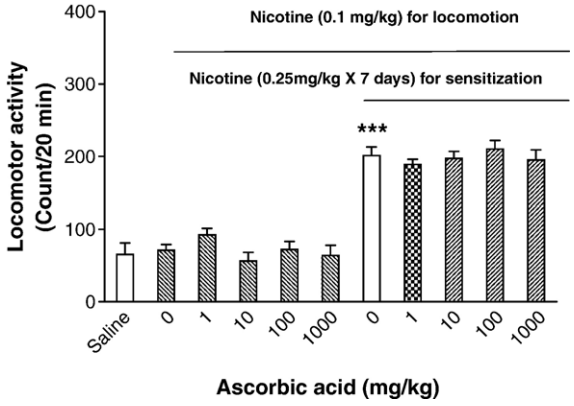

Fig. 6. Effects of ascorbic acid on the expression of nicotine-induced behavioral sensitization. When the animals received ascorbic acid (1, 10, 100 and 1000 mg/kg, i.p.), 20 min before nicotine challenge dose (0.1 mg/kg) in the test day, the expression of the nicotine-induced behavioral sensitization did not change. Each point is the mean±S.E.M. for 7–9 mice. ***$P<0.001$ compared with saline control group.

ascorbic acid effect: $F(11,95)=6.43$, $P<0.001$, nicotine effect: $F(1, 95)=5.63$, $P<0.001$, ascorbic acid X nicotine: $F(11, 95)=6.54$, $P<0.001$].

However, when the animals received ascorbic acid (1, 10, 100 and 1000 mg/kg, i.p.), 20 min before a nicotine challenge dose of (0.1 mg/kg) on the test day, the expression of the nicotine-induced behavioral sensitization did not change [two-way ANOVA; within-group comparison: ascorbic acid effect: $F(11,93)=0.83$, $P>0.05$, nicotine effect: $F(1, 93)=8.12$, $P<0.001$, ascorbic acid X nicotine: $F(11,93)=1.21$, $P>0.05$] (Fig. 6).

4. Discussion

The major finding of the present study is that ascorbic acid administration could interact with nicotine-induced place conditioning as well as nicotine-induced behavioral sensitization in mice.

Our results may support previous findings concerning nicotine induced place preference (Zarrindast et al., 2003; Sahraei et al., 2004) and behavioral sensitization (Biala, 2003) in mice. Several studies confirm that nicotine-induced place preference is based on the activation of the mesocorticolimbic dopamine system. This system is regarded as the major substrate of reward and reinforcement for nicotine as well as other drugs of abuse (Di Chiara, 2000). Nicotine could induce dopamine release in nucleus accumbens, which accounts for its rewarding as well as psychomotor stimulating effects (Wonnacott et al., 2005; Cadoni and Di Chiara, 2000; Balfour et al., 1998; Brazell et al., 1990). However, it also activates endogenous opioid system (See: Pomerleau, 1998 for Rev) and nitric oxide (Sahraei et al., 2004; Shim et al., 2002; Vleeming et al., 2002) as well, and investigators emphasize a role for these systems in mediation of nicotine effects.

Administration of ascorbic acid neither changes the time spent in the drug-paired compartment in the place-conditioning paradigm nor did it induce locomotor activity. There are no data concerning the effects of ascorbic acid on the place-conditioning paradigm. However, several studies have shown the interaction of ascorbic acid with the dopaminergic system in the nucleus accumbens (Debler et al., 1988; Pierce et al., 1995; Gu et al., 2005) and the striatum (Heikkila et al., 1981; Gu et al., 2006). More over, local applications of ascorbic acid enhanced the response of neurons to dopamine (Tolbert et al., 1992) and glutamate (Rice, 2000). In addition, ascorbic acid acts as a dopamine antagonist in the brain and also inhibits the binding of radio labeled dopamine agonists and antagonists in the brain tissues (Tolbert et al., 1992). Considering the effects of ascorbic acid on dopamine receptors and dopamine function (Tolbert et al., 1992; Pierce et al., 1995; Rice, 2000), one may anticipate that the drug administration may produce conditioned place aversion and reduce locomotor activity. However, in the present study tendencies for saline-paired compartment was observed in some doses of ascorbic acid but no place aversion was observed in the animals that received ascorbic acid. Moreover, no changes in locomotor activity were detected following ascorbic acid administration, which may also confirm the neuromodulatory role for ascorbic acid (Rebec and Pierce, 1994).

In the second part of the experiments, administration of ascorbic acid inhibits both the acquisition and expression of nicotine-induced conditioned place preference. The effects were not dose-dependent. The effect of ascorbic acid on nicotine-induced conditioned place preference is not well studied. However, nicotine releases dopamine in the mesolimbic dopamine system for inducing its rewarding effects (Wonnacott et al., 2005) was it also stimulates dopamine release via *n*-methyle-D-aspartate (NMDA) receptors located in the ventral tegmental area (Fu et al., 2000), which indicated a potential role for glutamate system in nicotine-induced reward. On the other hand, ascorbic acid administration has been shown to reduce dopamine and glutamate function within the central nervous system (de Angelis, 1995; Pierce et al., 1995; Rice, 2000). Based on these findings, it can be concluded that ascorbic acid may interfere with the nicotine effects via reduction of dopamine and/or glutamate activity in the brain (Rebec and Pierce, 1994). On the other hand, glutamate *N*-methyle-D-asparatate (NMDA) receptor activity increases nitric oxide release (Garthwaite et al., 1989). Some data exist that imply a role for nitric oxide in nicotine place conditioning (Sahraei et al., 2004) as well as nicotine dependence (Vleeming et al., 2002). Moreover, ascorbic acid can inhibit nitric oxide production induced by nicotine in human renal cells (Halimi and Mimran, 2000), which may be true in the brain as well. Taken together, ascorbic acid may inhibit nicotine-induced conditioned place preference by inhibition of nicotine-induced nitric oxide production. One important neurotransmitter mechanism for mediation of nicotine dependence is the induction of the endogenous opioid system (Pomerleau, 1998). However, since it is not clear that ascorbic acid has any effect on endogenous opioid system, it is difficult for any decision on this matter. In contrast to our results ascorbic acid potentiates amphetamine-induced conditioned place preference in rats (Pierce et al., 1995), which strain and sex difference may explain the discrepancy. In addition, differences between nicotine and amphetamine action could be considered as another cause of differences in these results. Moreover, the influence of ascorbic acid on nicotine metabolism must be

considered in this regard especially for chronic (acquisition) treatments (Dawson et al., 1999; Kelly, 2003). Another possible mechanism, especially for higher dose of ascorbic acid may be hypertonicity due to the drug.

In agreement with a previous report (Picciotto, 2003), the present data showed that acute injection of nicotine to animals reduced locomotor activity. The effect was dose-independent. However, acute administration of ascorbic acid did not change the animal's activity, which is also in agreement with other findings in this regard (Miquel et al., 1999).

Our results indicate that repeated injections of nicotine produced behavioral sensitization, which are in agreement with some data obtained by others (Biala, 2003), but are not supported by some investigators, which emphasize that nicotine has an inhibitory effect in mice (For review see: Picciotto, 2003). The response is not dose-dependent To our knowledge, it is difficult to explain why the drug's effects are not dose-dependent. One explanation, however, is that: the behavioral effects produced following peripheral administration of nicotine result from the ability of nicotine to stimulate antagonistic pathways in the central nervous system through several nicotinic acetylcholine receptor subtypes that possess different sensitivities to activation and desensitization by nicotine. Thus, small differences in the activation state, connectivity or sensitivity of neuronal pathways among individuals could result in large differences in the behavioral responses produced by nicotine (Picciotto, 2003). Nicotine sensitization has been postulated to be mediated by dopamine in the core portion of nucleus accumbens (Cadoni and Di Chiara, 2000; Di Chiara, 2000). There is evidence that dopamine and glutamate neurons within the ventral tegmental area and nucleus accumbens (Wonnacott et al., 2005; Fu et al., 2000; Balfour et al., 1998) are the target neurons for the action of nicotine.

Injection of ascorbic acid during development of sensitization reduced the effects of nicotine on locomotor activity. Nicotine sensitization may depend upon several mechanisms, which are not fully understood. Changes in mesoaccumbens dopamine (Di Chiara, 2000), glutamate system (Fu et al., 2000) and nitric oxide activity (Shim et al., 2002) all seems to be influenced by nicotine. Since ascorbic acid can interact with dopamine (Pierce et al., 1995; de Angelis, 1995) and glutamate (Rice, 2000) systems and also the drug acts as an antioxidant in the brain (Rice, 2000; Rebec and Wang, 2001), which reduces nitric oxide production, it may be postulated that ascorbic acid reduces nicotine effects via these mechanisms. In addition, the effects of hypertonicity should be considered. Another explanation is that the effects were observed following ascorbic acid administration could be due to the anti-oxidant activity of the drug. However, for assessing this possibility, we recommend that the effects of another antioxidant compound such as *N*-acethyle-cisteine could be examined in future experiments.

In conclusion, the present study confirms and extends the previous studies concerning the effects of ascorbic acid on the rewarding and sensitization properties of nicotine and is well in agreement with the idea that ascorbic acid elicits a neuromodulation with in the nervous system (Rebec and Pierce, 1994).

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