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Ascorbic Acid Antagonizes Ethanol-Induced Locomotor Activity in the Open-Field

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MIQUEL, M., M. A. AGUILAR AND C. M. G. ARAGÓN. *Ascorbic acid antagonizes ethanol-induced locomotor activity in the open field.* PHARMACOL BIOCHEM BEHAV **62**(2) 361–366, 1999.—It has been reported that ascorbic acid (AA) antagonizes the physiological and behavioral effects of dopamine (DA). AA reduces locomotor activity induced by dopaminergic agonist drugs. Also, AA amplifies the action of antidopaminergic drugs. Ethanol, like other drugs, produces a release of DA in the mesolimbic pathway, and at some doses, induces locomotor activity in mice. The ethanol-induced locomotor activity could be dopamine-dependent because it can be reduced by antidopaminergic drugs. In the present study, we investigated whether an acute administration of AA reduces ethanol-induced locomotor behavior. AA, at doses (0.0, 21.85, 87.5, 175, 350, and 1400 mg/kg) was injected IP into mice, 0, 30, 60, or 90 min before an IP injection of ethanol (0.0, 0.8, 1.6, 2.4, and 3.2 g/kg). Locomotor activity was evaluated in open-field chambers. Our results showed that AA (350 and 1400 mg/kg) reduced ethanol-induced locomotor activity when injected 30 min before ethanol treatment. This effect was lost when ethanol was administered 90 min after AA injection. AA also reduced locomotor activity produced by *d*-amphetamine and methanol. The results support a pro-dopaminergic action of ethanol, and suggest a common dopaminergic pathway for the drugs of abuse in locomotor activity. © 1999 Elsevier Science Inc.

Ascorbic acid Ethanol Methanol *d*-Amphetamine Dopamine Locomotor activity Blood ethanol levels

MESOLIMBIC dopaminergic pathways have been implicated in the control over locomotion in incentive and novel situations (10,36). For example, it has been shown that drugs (e.g., *d*-amphetamine, methamphetamine, cocaine, and morphine) increase locomotor activity in the open field (10,22,24,36) by activating mesolimbic dopaminergic pathways. Ethanol, like the above drugs, produces activation of DA systems in the brain and also induces locomotor activity. Thus, ethanol, in a dose-dependent manner, enhances the firing rate of dopamine neurons in the striatum and nucleus accumbens (5,16,23), besides stimulating dopamine turnover, metabolism, and release (4,12,24,28). Likewise, ethanol, at some doses, induces locomotor activity in the open field in mice (1,2,8,14). Previous studies have also indicated that the dopamine function could be involved in the mediation of ethanol-induced locomotion (4,8,12,28). For example, apomorphine, an agonist of presynaptic dopamine receptors, decreased ethanol-induced locomotor behavior, probably by preventing the release of dopamine induced by ethanol (12). Furthermore, the stimulating effect of ethanol on locomotor activity was also prevented by postsynaptic dopamine antagonists such as haloperidol (8,27,28), tiapride (8), SCH-23390 (8,28), and raclopide (28). It has also been reported that coadministration of SCH-23390 plus raclopide produced a higher decrease in locomotor activity induced by ethanol than administration of one antagonist alone (28). These experimental results suggest that ethanol-induced locomotor behavior could be affected by substances that affect the dopaminergic function.

Ascorbic acid (AA) is a vitamin with antioxidant properties that the brain accumulates from the blood supply and maintains at relatively high concentrations (26). External administration of AA produces a wide range of effects on organisms (26). One of the known effects on the CNS is its antidopaminergic effect. In most experimental conditions, AA antagonizes the physiological and behavioral effects of the dopamine function (DA).

In relation to the physiological effects of AA on the dopamine function, several studies have demonstrated that AA inhibits the binding of radiolabeled dopamine agonists

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and antagonists in brain tissue homogenates (19,22,30,35). It has been suggested that this effect could be produced by means of the direct action of AA on DA receptors (22,30). Likewise, AA also attenuates the ability that some drugs have to release DA in the striatum and nucleus accumbens. For example, preadministration of AA reduces the depletion of DA induced by methamphetamine and 1-methyl-4-phenyl pyridinium (Mpp+) (33,34). At present, these physiological effects are not well understood, but all of them imply an antagonism by AA on the dopaminergic function.

In addition to affecting the physiological dopaminergic function, several reports have demonstrated that AA reduces behavioral changes induced by dopaminergic agonist drugs. Pretreatment with AA (500–2000 mg/kg) blocked *d*-amphetamine–induced stereotype (32) and locomotor activity (19) in mice. Besides, AA pretreatment reduces ipsilateral turning behavior produced by *d*-amphetamine in rats with unilateral lesions of the nigrostriatal dopaminergic pathway (31). Other studies have shown that AA amplifies the action of antidopaminergic drugs. Administration of AA (1000 mg/kg) increased haloperidol's ability to block *d*-amphetamine–induced motor behavior and haloperidol-induced catalepsy (17). Likewise, the antipsychotic effect of antidopaminergic drugs was also enhanced by pretreatment with AA in the psychosis produced by phencyclidine (17).

Therefore, preadministration of AA should be able to attenuate the increase in locomotor activity produced by the acute administration of ethanol. The present study was conducted to investigate whether AA changes ethanol-induced locomotor behavior. Because AA works as an antidopaminergic drug, we think that this study could add to our knowledge about what common mechanism is shared by ethanol and other drugs of abuse. In this study, we report that AA blocks locomotion induced by ethanol in the open field in a time- and dose-dependent manner.

METHOD

Animals

Male Swiss–Webster mice (32–42 g) purchased from Harlan Interfauna Ibérica (Barcelona, Spain) were used in this study. Animals were housed in groups of four per cage, with food and water available ad lib. The mice were allowed 1 week to adapt to the animal colony prior to experimentation. The colony was maintained at 22°C, with lights on from 08:00 to 20:00 h. Testing was always conducted between 10:30 and 13:00 h. All experimental procedures complied with European Community Council Directive (24 November 1986) (86/609/ECC) for the use of animal subjects.

Drugs

Ascorbic acid and *d*-amphetamine were purchased from Sigma-Aldrich Quimica, S.A. (Spain). Ethanol and methanol solutions (Panreac Quimica, S.A., Spain) were diluted at 20% v/v from a 96% solution. All drugs were prepared in distilled water. Injections were administered IP.

Apparatus

The open field chambers have been described previously (1,2). Briefly, the chamber consisted of a clear glass cylinder 25 cm in diameter and 30 cm high. The floor of the cylinder was divided into four equal quadrants by two intersecting lines drawn on the floor. A locomotion score was assigned each time an animal crossed over from one quadrant to another with all four legs. The test room was illuminated with soft white light.

Procedure

To evaluate the effects of AA on ethanol-induced locomotion, three experiments were carried out. In the first experiment, subjects received injections of either AA (350 mg/kg/10 ml) or saline (S) 30 min prior to the open-field testing. Immediately before testing, mice received an injection of one of these doses of ethanol: 0.0, 0.8, 1.6, 2.4, or 3.2 g/kg. In the second experiment, five groups of mice received injections of AA (21.85, 87.5, 175, 350, or 1400 mg/kg/10 ml) 30 min before an injection of 2.4 g/kg of ethanol. To analyze the time course of AA effects, a third experiment was designed in which three groups of mice were treated with AA (350 mg/kg), simultaneously, 60 or 90 min. before the administration of 2.4 g/kg of ethanol. Following ethanol injections, mice were placed individually in the open-field apparatus for 20 min. Locomotor activity was recorded for the last 10 min.

In another study, the effects of AA (350 mg/kg/10 ml) on *d*-amphetamine (2 mg/kg/10 ml) and methanol-induced locomotion (2.4 g/kg) were analyzed. Mice were challenged with an AA injection 30 min before *d*-amphetamine or methanol injections. Locomotor activity was evaluated by the same procedure as in the experiments mentioned above.

Blood Ethanol Assays

To analyze ethanol levels in the blood, mice were treated with AA (350 mg/kg/10 ml) or saline 30 min before an ethanol injection (2.4 g/kg) and then truncal blood was collected 15, 30, and 60 min after ethanol treatment. Blood ethanol levels were enzymatically determined with an Alcohol Diagnosis Kit from Sigma-Aldrich Quimica, S.A. (Spain).

Statistical Analysis

All data were analyzed by means of two-way ANOVAs. Fisher's tests were performed to evaluate the differences between means. The statistical computer programme Systat.5.2. was used in this study.

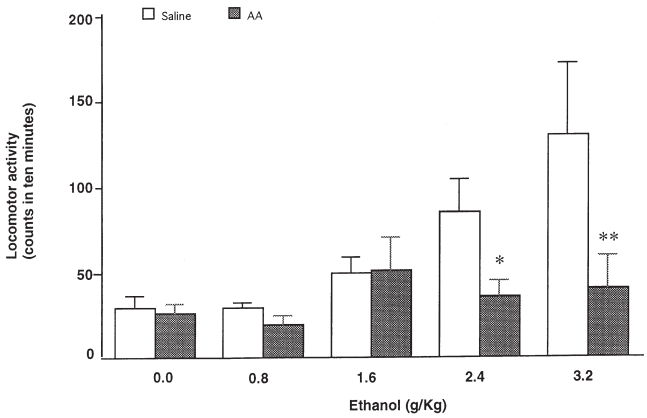

FIG. 1. Effect of ascorbic acid or saline on ethanol-induced locomotor activity. Mean ± SEM locomotor activity (counts in 10 min) for all treatment groups ($n = 8$). Mice were pretreated IP with saline or ascorbic acid (350 mg/kg/10 ml) 30 min before ethanol (0.0, 1.6, 2.4, and 3.2 g/kg) (**$p < 0.01$; *$p < 0.05$).

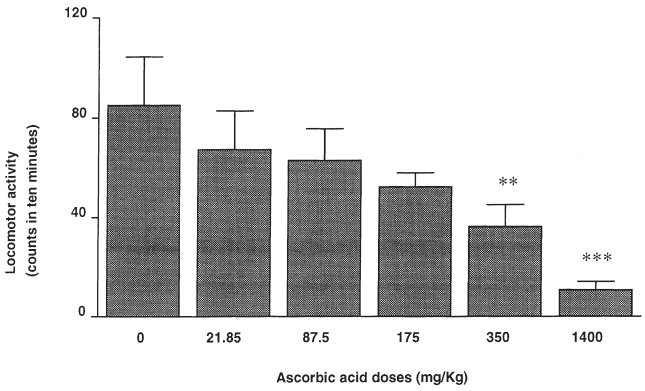

FIG. 2. Effect of different doses of ascorbic acid on locomotion induced by ethanol. Mean ± SEM locomotor activity (counts in 10 min) for all treatment groups ($n = 8$). Mice were pretreated IP with saline or ascorbic acid (21.85, 87.5, 175, 350, and 1400 mg/kg/10 ml). Thirty minutes after this treatment, ethanol (2.4 g/kg) was administered to mice. (***$p < 0.001$; **$p < 0.01$).

RESULTS

Figure 1 represents the effect of AA on ethanol-induced changes in locomotor activity. A two-way analysis of variance (ANOVA) revealed a significant effect for ethanol treatment, $F(4, 70) = 4.01$, $p < 0.01$, a significant effect for AA pretreatment, $F(1, 70) = 7.32$, $p < 0.01$, and a significant AA–ethanol interaction, $F(4, 70) = 2.43$, $p < 0.05$. Comparisons were made of pairs using Fisher's test. Ethanol had a biphasic effect on locomotor activity increasing crossings significantly at 2.4 and 3.2 g/kg. AA administration alone did not produce effects on locomotor activity ($p > 0.05$). However, animals pretreated with AA and ethanol had significantly lower locomotion counts compared to animals pretreated with saline and then with ethanol at 2.4 ($p < 0.05$) and 3.2 g/kg ($p < 0.01$).

Data for the effect of different doses of AA on hyperactivity induced by 2.4 g/kg of ethanol are displayed in Fig. 2. A one-way ANOVA showed effect of AA pretreatment to be significant, $F(5, 41) = 3.22$, $p < 0.01$. Fisher's test for pairwise comparison demonstrated a dose-dependent effect of AA on locomotor behavior. At 350 and 1400 mg/kg AA produced a significant reduction of ethanol-induced locomotion ($p < 0.01$; $p < 0.001$, respectively).

In Fig. 3 the time course of the effect of AA on ethanol treated mice can be observed. A two-way ANOVA was only significant for AA pretreatment, $F(1, 56) = 6.35$, $p < 0.01$. No significant differences were found for the time effect, but a re-

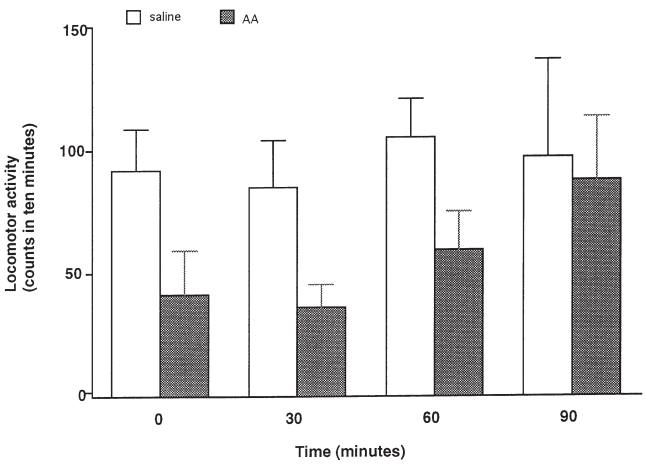

FIG. 3. Time course of ascorbic acid or saline effect on ethanol-induced locomotor activity. Mean ± SEM locomotor activity (counts in 10 min) for all treatment groups ($n = 8$). Mice were treated IP with saline or ascorbic acid (350 mg/kg/10 ml) simultaneously, or 30, 60, and 90 min before ethanol (2.4 g/kg).

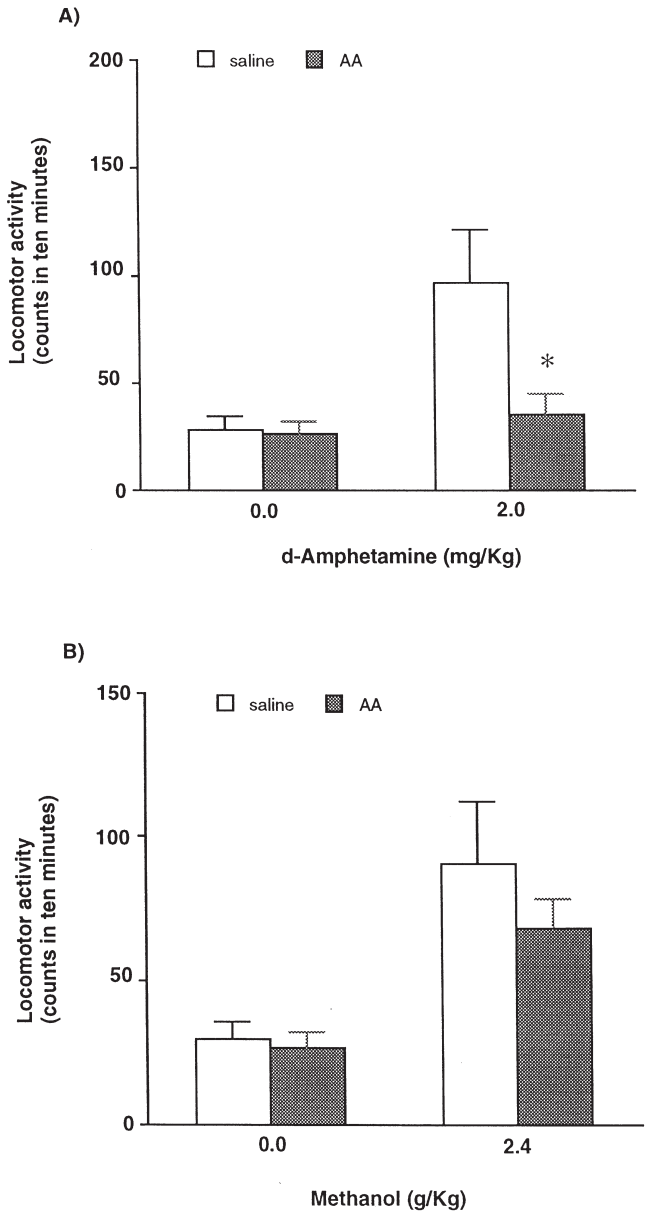

FIG. 4. (A) Effect of ascorbic acid or saline on *d*-amphetamine–induced locomotor activity. Mean ± SEM locomotor activity (counts in 10 min) for all treatment groups ($n = 8$). Mice were pretreated IP with saline or ascorbic acid (350 mg/kg) 30 min before *d*-amphetamine (2 mg/kg). (B) Effect of ascorbic acid or saline on methanol-induced locomotor activity. Mean ± SEM locomotor activity (counts in 10 min) for all treatment groups ($n = 8$). Mice were pretreated IP with saline or ascorbic acid (350 mg/kg) 30 min before methanol (2.4 g/kg).

duction on locomotor activity can be observed until 90 min after injection of AA.

Figure 4 shows the effect of pretreatment with AA on *d*-amphetamine–induced (A) and methanol-induced (B) locomotor activity. For *d*-amphetamine, a two-way ANOVA revealed a significant effect for *d*-amphetamine treatment, $F(1, 28) = 7.58$, $p < 0.01$, a significant effect for AA pretreatment, $F(1, 28) = 5.18$, $p < 0.05$, and a significant effect for AA–*d*-amphetamine interaction, $F(1, 28) = 4.51$, $p < 0.05$. *d*-Amphetamine–induced locomotor activity was significant blocked by AA administration. For methanol treatment, a two-way ANOVA revealed a significant effect for methanol treatment, $F(1, 28) = 16.46$, $p < 0.001$, but for neither AA pretreatment, $F(1, 28) = 1.06$, $p > 0.05$, nor AA–methanol interaction, $F(1, 28) = 0.59$, $p > 0.05$. Methanol increased locomotor activity and the pretreatment with AA attenuated this induction on locomotor activity, but no significant reduction was observed.

In Table 1 blood ethanol levels are shown for saline and AA pretreated mice. A two-way ANOVA displayed a significant effect for AA pretreatment, $F(1, 30) = 37.96$, $p < 0.01$, also for the time factor, $F(2, 30) = 35.31$, $p < 0.01$, but not for AA–time interaction, $F(2, 30) = 0.18$, $p > 0.05$. Higher blood ethanol levels were observed in animals treated with AA at all doses and test times.

DISCUSSION

Findings from the present study support a common mechanism for the stimulating effects of ethanol and other drugs of abuse in locomotor activity. Our results demonstrated that ethanol produced a stimulatory effect on locomotor activity that was blocked by AA in a time- and dose-dependent manner. AA reversed this stimulatory effect when it was administered at a dose of 350 and 1400 mg/kg 30 min before ethanol treatment. At 90 min after injection of AA its effect seemed to decline, because a recovery of locomotor activity to control levels was observed. The same treatment with AA (350 mg/kg) also reduced locomotor activity induced by *d*-amphetamine and methanol.

Although we detected a delay in metabolic ethanol elimination with AA treatment, a pharmacokinetic process does not seem to explain the AA antagonism on effects of ethanol on locomotion. An increase in blood ethanol levels should produce a greater stimulating effect. However, our data showed lower locomotor activity in AA-treated mice than in saline-treated mice. The mechanism by which AA delayed the rate of ethanol clearance is unknown, but should be unrelated to behavioral AA effects. In contrast to our study, some authors have found out that AA did not change (15), or accelerated (9,29,39) the disappearance of ethanol.

Some prior studies have also described an interaction between AA and ethanol-induced behavior (6,15,38) in mice. In two studies (15,38), it has been demonstrated that AA enhanced sleeping time in a large range of doses (430–1720 mg/kg) when it is administered 30 min before ethanol. The data from these studies seem to point out that AA increased ethanol-induced depression. The mechanism behind the AA–ethanol interaction in this depressant effects is unknown. Nevertheless, some studies have suggested a role of dopaminergic systems in this interaction (8,17,38). Cohen et al. (8) found an increase in ethanol-induced sleeping time with haloperidol, whose behavioral effects have been shown to be enhanced by AA (11). Furthermore, AA reduced apomorphine-induced locomotor activity as well as increasing ethanol sleeping time at the same doses (38). The data from the present study also showed a reduction of a stimulatory effect on locomotor activity by AA. However, some contradictory aspects remain without explanation, because the effect of AA seems paradoxical. Thus, it has been displayed that AA lengthened the onset of narcosis at higher doses (1000 and 1500 mg/kg) (15), diminished ethanol-induced ataxia (15), and prevented swimming impairment due to ethanol in mice (6). Therefore, AA enhanced a depressant-ethanol effect (sleeping time), and reduced its stimulating effect (locomotion), along with antagonized ataxia, motor impairment, and delayed the onset of sleeping time, all of which are effects that show an attenuation of the depressant actions of ethanol.

Several experimental pieces of research have shown that, in the same way as *d*-amphetamine (10,25,30), the stimulating effect of ethanol on locomotion is dopamine-dependent (8,12, 27,28). It is interesting to note that with a similar range of AA doses (350–1400 mg/kg), our findings for ethanol parallel previous studies carried out with dopaminergic agonist drugs (19,31). A reduction of *d*-amphetamine–induced motor activity produced by AA in mice (19,30–32) has, therefore, been displayed. Similarly, it has been described that AA (1000 mg/kg) antagonized ipsilateral turning behavior produced by *d*-amphetamine in rats with unilateral lesions of the nigrostriatal dopaminergic pathway (31). AA pretreatment also blocked *d*-amphetamine–induced locomotion in the open field (19) and stereotypy (32).

In addition to these studies, in the present work, we replicated the effects of AA on locomotor behavior produced by *d*-amphetamine in the open field. Moreover, the antidopaminergic effect of AA was supported in our study by demonstrating that methanol-induced locomotor activity was also reduced by AA. Methanol is another alcohol that is able to increase extracellular DA in the striatum and nucleus accumbens, although, as a microdialysis study showed, its relative potency to produce DA release is lower than that of ethanol (37). Therefore, all of these experimental results demonstrate

TABLE 1

EFFECT OF ASCORBIC ACID TREATMENT ON BLOOD ETHANOL LEVELS

		Blood Ethanol (mg/dl)		
Group	*n*	15 min	30 min	60 min
Saline-ethanol	6	242.14 ± 5.07	190.32 ± 17.86	153.32 ± 14.69
AA-ethanol	6	301.66 ± 7.55†	237.50 ± 5.58*	210.49 ± 7.64†

AA; 350 mg/kg, 30 min before ethanol injection; 2.4 g/kg.
*$p < 0.05$.
†$p < 0.01$.

that AA, at the doses reported above, could behave in a similar way to dopaminergic antagonist drugs.

Although the mechanism for the interaction between AA and ethanol-induced locomotion is unknown, these earlier findings, together with our present results, suggested dopamine mesolimbic pathways as a locus for interaction between AA and ethanol-induced behaviors. However, because the dopamine function is regulated by other neurotransmitters, it has been claimed that AA could exert its antidopaminergic action (26) indirectly by means of other neurotransmitter systems. For example, a postsynaptic interaction between dopaminergic and glutamergic neurons in the neostriatum has been described (14). In fact, AA can increase glutamergic neurotransmission (13). Glutamate has also been described as having opposed effects to DA in the neostriatal function (7). Likewise, it has been reported that ethanol, in acute administration, antagonized the glutamergic function (20). Therefore, AA–ethanol interactions could also be taking place in a glutamergic locus (26).

In conclusion, we showed that AA, in our open-field paradigm, blocked ethanol's stimulatory effect on locomotor activity at the same doses as it also blocked methanol and *d*-amphetamine–induced locomotor behavior. Although earlier studies had described that AA enhanced ethanol-induced depression, to date no data about AA affecting ethanol-induced locomotor activity in the open-field had been reported. The present data support the mesolimbic dopaminergic systems as putative loci of AA and ethanol interactions, either directly or through other neurotransmitter systems.

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