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Effects of L-Ascorbic Acid on Ethanol-Induced Central Nervous System Depression in Mice

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FERKO, A P *Effects of L-ascorbic acid on ethanol-induced central nervous system depression in mice* PHARMACOL BIOCHEM BEHAV 24(3) 543–547, 1986 —Male Swiss-Webster mice were administered ethanol immediately before a motor coordination test Controls and animals treated with 1, 2 or 3 g/kg, IP, of ethanol remained on a suspended meter stick for 240±0, 232±8, 93±2 and 75±5 sec, respectively Blood ethanol levels at the end of the test period (4 min) or when the animal fell from the meter stick were 1 02±0 03, 2 13±0 09 and 2 24±0 07 mg/ml for the 1, 2 and 3 g/kg dose of ethanol, respectively Thirty min prior to ethanol (2 g/kg, IP) animals received L-ascorbic acid in doses of 500 or 1000 mg/kg, IP Both doses of L-ascorbic acid significantly enhanced the duration of time that the animals spent on the meter stick When animals were given 1 g/kg, IP, of ethanol their rate of walking (cm/min) on the meter stick was significantly increased over controls Administration of L-ascorbic acid (1000 mg/kg, IP) 30 min prior to ethanol (1 g/kg) did not change the rate of locomotion In experiments on ethanol-induced hypnosis (sleep-time), animals received L-ascorbic acid (250, 500, 1000 or 1500 mg/kg, IP) or saline 30 min prior to ethanol (4 g/kg, IP) L-ascorbic acid increased the time of onset of hypnosis significantly at doses of 1000 and 1500 mg/kg With these doses of L-ascorbic acid sleep duration and blood ethanol content were not altered L-ascorbic acid, however, increased ethanol-induced hypnosis at a dose of 500 mg/kg These results indicated that L-ascorbic acid attenuated some of the depressant effects of ethanol, but not the stimulatory effect from a low dose of ethanol It is possible that the ethanol-ascorbic acid interaction may involve a dopaminergic mechanism

L-ascorbic acid Ethanol-induced depression

THE administration of ethanol to animals can induce alterations in behavior Small doses of ethanol produce an activating effect on locomotion while larger doses cause a depressant effect [21] In the literature also reports indicate the depressant effect of ethanol on motor coordination [7, 8, 11] Another approach to study the central depressant action of ethanol is to use a dose of ethanol that induces hypnosis or sleep in animals This type of experiment is relatively common and has been used in investigations studying the phenomenon of tolerance to ethanol [21]

Although there are many investigations on the behavioral effects of ethanol, there is limited information on agents that reduce or antagonize the central depressant properties of ethanol However, one such report that is present in the literature involves sodium ascorbate and ethanol Sodium ascorbate antagonizes ethanol-induced impairment of swimming in mice when sodium ascorbate is given along with ethanol administration [3]

It would be of interest to examine further the effect of ascorbic acid on ethanol-induced behavioral responses Therefore, this present study investigates the interaction between ethanol and L-ascorbic acid on motor coordination and ethanol-induced hypnosis Blood ethanol concentrations are determined in mice when ethanol is administered alone and in combination with L-ascorbic acid

METHOD

Male Swiss-Webster mice (28–32 g) were obtained from Perfection Breeders (Douglasville, PA) and were housed for 1 week prior to experimentation at 22±1°C with a light cycle from 6 00 a m to 6 00 p m The animals had free access to Purina Laboratory Chow (Ralston Purina Co , St Louis, MO) and water, however, they were fasted 18 hr prior to drug or saline administration but water was available ad lib The bedding used in the cages was ground corn cob ⅛ in (Anderson Cob Division, Maumee, OH) Ethanol solutions for injection were prepared from 95% ethanol L-ascorbic acid (Baker Analyzed Reagent) was obtained from Thomas Scientific, Swedesboro, NJ Solutions of L-ascorbic acid were prepared under nitrogen gas, adjusted to pH 6 8 with NaOH solution (2 N) and placed in a light-resistant container

Motor Coordination

A meter stick (2 m long) was suspended 23 cm above the laboratory counter. Foam rubber pads were placed under the meter stick To test for motor coordination mice were placed on the narrow portion of the meter stick (8 mm) Measurements were taken for the time spent on the meter stick and the distance walked Test time was 240 sec Mice received

TABLE 1

EFFECT OF L-ASCORBIC ACID (AA) TO REDUCE ETHANOL (ETOH 2 g/kg, IP) INDUCED ATAXIA

Control			Ethanol-Treated			
Group	N	Time (sec)	Group	N	Time (sec)	Blood ETOH (mg/ml)
SAL + SAL	14	240 ± 0 0*	SAL + ETOH	10	93 ± 2†	2 13 ± 0 09
AA (500 mg/kg) + SAL	10	240 ± 0 0	AA (500 mg/kg) + ETOH	11	147 ± 16†§	1 95 ± 0 06
AA (1000 mg/kg + SAL	10	240 ± 0 0	AA (1000 mg/kg) + ETOH	12	194 ± 16‡¶	2 03 ± 0 05

L-Ascorbic acid or saline (SAL) are given (IP) 30 min prior to ethanol Duration of test is 240 sec
*Mean ± S E M
†Significantly different from corresponding control ($p<0$ 01)
‡Significantly different from corresponding control ($p<0$ 05)
§Significantly different from saline + ETOH ($p<0$ 05)
¶Significantly different from saline + ETOH ($p<0$ 01)

TABLE 2

EFFECT OF ETHANOL ALONE (ETOH, 1 g/kg, IP) ON WALKING (cm/MIN) ON THE METER STICK AND THE EFFECT OF ETHANOL WITH L-ASCORBIC ACID (AA, 1000 mg/kg, IP)

Control			Ethanol-Treated			
Group	N	Locomotion (cm/min)	Group	N	Locomotion (cm/min)	Blood ETOH (mg/ml)
SAL*† + SAL	14	220 ± 17‡	SAL† + ETOH	11	344 ± 24§	1 05 ± 0 03
AA† + SAL	10	154 ± 20¶	AA† + ETOH	9	327 ± 19§	1 12 ± 0 05

*SAL=saline, IP
†Administered 30 min prior to second injection
‡Mean ± S E M
§Significantly different corresponding control ($p<0$ 01)
¶N S

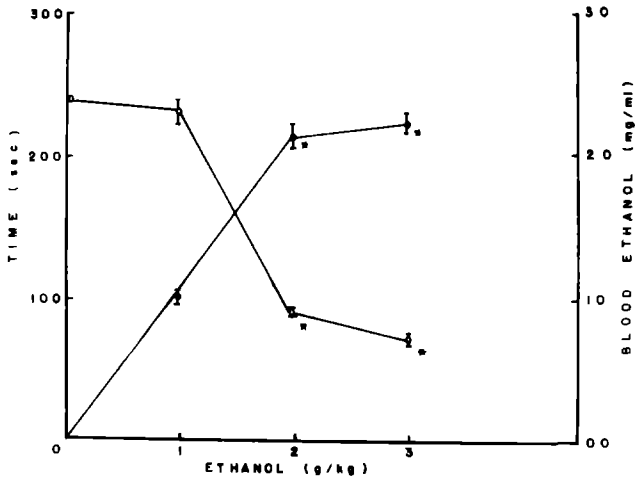

FIG 1 The effect of acute ethanol (1, 2, or 3 g/kg, IP) to alter motor coordination as assessed by time (sec) spent on meter stick (○—○) Blood ethanol levels (●—●) were obtained at the end of the test period (240 sec) or when the animal fell from the meter stick Each value represents the mean±S E M of 8 to 14 mice *$p<0$ 05

saline (0 9% NaCl) or L-ascorbic acid solution 30 min prior to injection of saline or ethanol solution Immediately after the administration of saline or ethanol the mice were tested for motor coordination on the meter stick Concentrations of drug solutions were prepared so that animals received 0 02 ml/g In the motor coordination experiment doses of ethanol were 1, 2 or 3 g/kg, IP and doses of L-ascorbic acid were 500 or 1000 mg/kg, IP From each animal immediately after testing, blood samples (20 μl) were obtained from the orbital sinus to determine blood ethanol concentrations according to an enzymatic method [12]

Ethanol-Induced Hypnosis

Sleep time was used as an index of ethanol-induced central nervous system depression and was measured as the time interval between the loss of the righting reflex after ethanol injection (4 g/kg, IP) and the gain of the righting reflex The gain of the righting reflex required that the animal be able to re-right himself 3 times within 1 min, after again being placed on his back In addition the onset of hypnosis (time between ethanol injection and loss of the righting reflex) was recorded

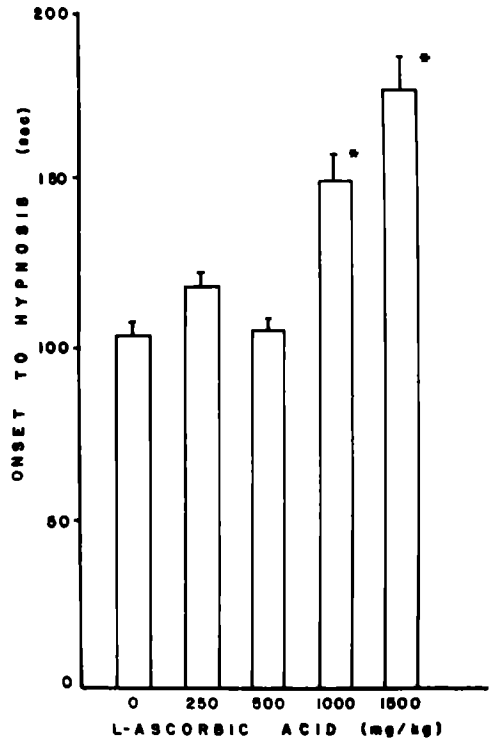

FIG 2 Effect of L-ascorbic acid on latent period to ethanol-induced hypnosis (sleep-time) L-ascorbic acid is given 30 min prior to ethanol (4 g/kg, IP) Each value is the mean±S E M of 10 to 15 mice *$p<0$ 05

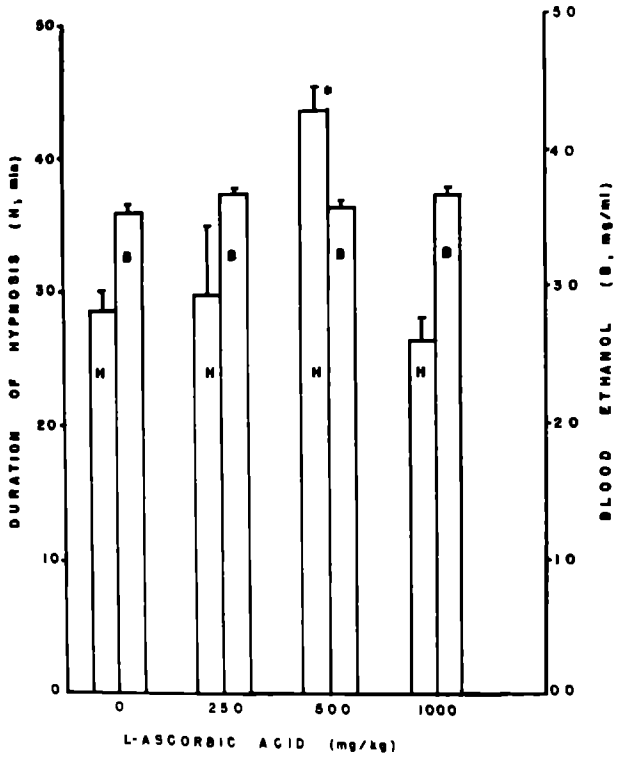

FIG 3 The effect of L-ascorbic acid on ethanol-induced hypnosis (sleep-time) L-ascorbic acid is given 30 min prior to ethanol (4 g/kg, IP) Each value represents the mean±S E M of 10 to 15 mice *$p<0$ 05

Mice received saline or L-ascorbic acid (250, 500, 1000 or 1500 mg/kg, IP) 30 min prior to injection of ethanol (4 g/kg, IP) Blood samples (20 μl) were taken from the orbital sinus of each animal when they regained the righting reflex

In another experiment mice were injected with saline or L-ascorbic acid 30 min prior to ethanol (4 g/kg, IP) At 3 and 10 min after ethanol administration blood samples (20 μl) were obtained from the orbital sinus of each animal to determine the blood ethanol content

Statistical Analysis

Significant differences were determined by analysis of variance (ANOVA) followed by Scheffe's Test [19] All data were analyzed using an Apple IIe Computer

RESULTS

The effects of ethanol on mice in the motor coordination test are illustrated in Fig 1 The duration of time spent on the meter stick was reduced significantly by ethanol at doses of 2 and 3 g/kg, IP A dose of 2 and 3 g/kg caused the mice to lose their balance and fall from the meter stick at 93±2 and 75±5 sec, respectively The time spent on the meter stick after the 2 and 3 g/kg dose of ethanol is significantly different from each other At a dose of 1 g/kg of ethanol all mice remained on the meter stick for 240 sec except one animal Blood ethanol levels of the mice also are shown in Fig 1

To study the interaction between ethanol and L-ascorbic acid on motor coordination the 2 g/kg dose of ethanol was used L-ascorbic acid administration prior to ethanol antagonized ethanol-induced ataxia (Table 1) At 500 and 1000 mg/kg L-ascorbic acid increased the duration of time that the mice remained on the meter stick by 58 and 104%, respectively Six out of 12 mice that received injections of L-ascorbic acid (1000 mg/kg) prior to ethanol dosage were able to stay on the meter stick for the duration of the test (240 sec) Blood ethanol concentrations also are listed in Table 1 In addition it should be mentioned that the various doses of L-ascorbic acid by themselves had no significant effect on the rate of walking that the mice did during the test

Although the 2 and 3 g/kg dose of ethanol induced ataxia in the mice, the 1 g/kg dose of ethanol seemed to have a stimulatory effect on mice This stimulatory effect of ethanol was manifested by an enhanced rate of walking on the meter stick during the 4 min test (Table 2) Ethanol (1 g/kg) increased the rate of walking by 56% when the data were compared with controls However, L-ascorbic acid (1000 mg/kg) failed to attentuate this stimulatory effect of ethanol (Table 2)

The second part of this study involved the effect of L-ascorbic acid on ethanol-induced sleep time (hypnosis) The results (Fig 2) indicated that L-ascorbic acid enhanced the time interval for the mice to lose their righting reflex from ethanol injection (4 g/kg, IP) L-ascorbic acid at doses of 1000 and 1500 mg/kg, IP increased the onset to sleep by 53 and 70%, respectively

In another experiment two groups of mice were injected IP with saline or L-ascorbic acid (1500 mg/kg) 30 min prior to

ethanol (4 g/kg, IP) and blood samples were obtained at 3 and 10 min The blood ethanol levels for the saline-ethanol group (N=6) were 3 96±0 08 and 3 80±0 08 mg/ml at 3 and 10 min, respectively The blood ethanol levels for the L-ascorbic acid-ethanol group (N=6) were 3 90±0 09 and 3 85±0 07 mg/ml at 3 and 10 min, respectively

The results for L-ascorbic acid on ethanol-induced hypnosis are shown in Fig 3 L-ascorbic acid (500 mg/kg) enhanced ethanol-induced hypnosis whereas other doses of L-ascorbic acid were without any effect Also blood ethanol concentrations that were determined in mice at the end of ethanol-induced hypnosis are indicated in Fig 3

The enhanced sleep-time from ethanol administration in the presence of ascorbic acid (500 mg/kg) appears to be a paradoxical effect since the other depressant actions of ethanol were antagonized by ascorbic acid The data obtained in these experiments support this finding However, further experiments using several different hypnotic doses of ethanol should be examined in the presence of ascorbic acid to confirm this effect of ascorbic acid (500 mg/kg) to enhance ethanol-induced hypnosis

The range of doses for ascorbic acid used in this study has been employed in previous work [3, 17, 23] The administration of ascorbic acid alone to animals caused no observable behavioral changes when they were compared with control animals Others also have reported no significant behavioral effects from IP injection of ascorbic acid at 1000 mg/kg [17] In addition the compound does not appear to be toxic even at high doses [6]

DISCUSSION

The results of the present study support an earlier finding [3] that ascorbic acid can attenuate the depressant effect of ethanol on motor coordination. Other experiments in this investigation show that ascorbic acid lengthens the time to the loss of the righting reflex after ethanol administration It does appear that ascorbic acid can antagonize some of the effects of ethanol In addition ascorbic acid increases the survival rate in mice that have received toxic doses of ethanol [24] In these present experiments ascorbic acid by itself produces no significant effect on behavior and these results agree with the reports of others [3,17]

One possible explanation for the observed effects of the ethanol-ascorbic acid interaction in mice might be related to altered pharmacokinetics of ethanol The data, however, indicate no significant differences in peak blood ethanol levels when they are determined at 3 and 10 min after ethanol administration alone or in the presence of ascorbic acid Further evidence on blood ethanol levels is presented in Fig 3 In addition others [24] have indicated that ascorbic acid does not delay the absorption or modify the distribution of ethanol Reports also show that ascorbic acid does not alter the disappearance of ethanol from blood in animals [24] and man [16] Although recent work [20] indicates that sodium ascorbate increases the biotransformation of ethanol by the catalase-H_2O_2 system under certain circumstances *in vitro*, the contribution of catalase in ethanol oxidation *in vivo* appears to be limited and possibly of no significant consequence after acute ethanol administration Since ascorbic acid is not altering the absorption or biotransformation of ethanol, the site for the ethanol-ascorbic acid interaction might be the central nervous system

In the mammalian brain high concentrations of ascorbate are present and the highest amounts of ascorbate are found in the amygdala, hippocampus and hypothalamus [14] The caudate and putamen also contain reasonable amounts of ascorbate Following IP injection ascorbic acid enters the central nervous system [23] Significant elevations of ascorbate in the brain occur within 15 min and remain for 2 hr The exact role that ascorbate has in central nervous system function is not fully known

Several reports have implicated ascorbic acid with the dopaminergic system in the brain Ascorbate inhibits dopamine-sensitive adenylate cyclase in mouse brain, decreases amphetamine-induced stereotype behavior that is mediated by the dopaminergic system, and reduces the hypothermic response from apomorphine injection [23] Similar to the effect of haloperidol, ascorbic acid attenuates the dopamine-induced cyclic AMP elevation in striatal tissue [22] Recent work on ascorbic acid and the behavioral response to haloperidol, demonstrates that ascorbic acid enhances the antidopaminergic effect of haloperidol [17] It has been suggested that ascorbic acid might reduce the effect of dopamine at the receptor level [17] while others have indicated that the effect of ascorbic acid to modulate dopamine receptors is unknown [13]

In the present study the effect of ethanol on motor coordination is inhibited and the onset to ethanol-induced hypnosis is lengthened by ascorbic acid There is evidence for a relationship between ethanol and the dopaminergic system Acute ethanol administration has been shown to release dopamine [18] and activate central dopaminergic neurons [4, 5, 9] The ethanol-induced impairment of performance in 6-hydroxydopamine treated animals is less than in controls [10] It has been suggested that dopamine neurons have a role in the intoxicating effect of ethanol [10] In animals the hypothermic response from ethanol injection may partially be mediated by a dopaminergic mechanism, since pimozide reduces this effect of ethanol [15] Therefore, it may be possible that when ascorbic acid antagonizes the effect of ethanol, ascorbic acid is acting in some manner to reduce the activity of the dopaminergic system

Although there is experimental evidence for a relationship between ascorbic acid and the dopaminergic system, the mechanism for the effect of ascorbic acid in the presence of ethanol may be more complex Other studies have suggested that ethanol at subhypnotic doses depresses the activity of dopaminergic neurons [1] and that pimozide enhances the depressant effect of ethanol on motor coordination [2] However, the dose of pimozide used in the study causes behavioral effects by itself The variant results that are reported in studies on ethanol and the dopaminergic system may be due to differences in experimental design, doses of drugs used, animal species and possibly other factors

In conclusion this present study has shown that ascorbic acid can attenuate some of the depressant effects of ethanol on behavior but does not antagonize the stimulatory effect of ethanol on locomotor activity Although some evidence suggest an association of ascorbic acid with the dopaminergic system, it seems that further work is required before a definitive explanation can be given for the ethanol-ascorbic interaction

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