THE INHIBITORY EFFECT OF ASCORBIC ACID ON APOMORPHINE-INDUCED LICKING BEHAVIOR IS MEDIATED BY DOPAMINE D2 RECEPTOR MECHANISMS IN RAT

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ABSTRACT

Ascorbic acid, an antioxidant vitamin, is found throughout the mammalian central nervous system (CNS). There is evidence that it may modulate neuronal activity, release of neurotransmitters and dopamine receptors activities. There are behavioral evidences supporting the antidopaminergic effect of ascorbic acid. This effect of ascorbic acid may in part modulates the stereotyped behaviors-induced by dopaminergic system. The purpose of the present study was to determine the interaction between ascorbic acid and the stereotyped licking behavior in rat. In the present study, effects of ascorbic acid and different dopamine receptor antagonists on apomorphine-induced licking behavior were examined. For the induction of licking, the dose of 0.5 mg/kg, S.C. of apomorphine was used and the number of licking was recorded over a 75 min period. Ascorbic acid (200-350 mg/kg, S.C.) dose-dependently reduced the licking behavior. Subcutaneous injection of ascorbic acid (250 mg/kg, ED61) potentates the inhibitory effect of dopamine D1 receptor antagonist, SCH 23390 (0.5 and 1 mg/kg, i.p.) but did not alter the inhibitory effect of dopamine D2 receptor antagonist, sulpiride (25 and 50 mg/kg, s.c.). These results suggest that the inhibitory effect of ascorbic acid on apomorphine-induced licking behavior is mediated by dopamine D2 receptor mechanisms.

KEYWORDS: Stereotyped behaviors, Licking, Ascorbic acid, Apomorphine, Rat

INTRODUCTION

Ascorbic acid, an antioxidant vitamin, is found throughout the mammalian central nervous system [1, 2]. Ascorbate, the endogenous from of vitamin C, is found in high concentration in the nigrostriatal system, where it appears to function as an extracellular neuromodulator [3]. Pretreatment with 500-1000 mg/kg ascorbate, for example, elevates striatal ascorbate in conjunction with an increase in striatal neuronal activity [4]. Repetitive licking behavior is a stereotyped phenomenon that is correlated with activation of the nigrostriatal system, is thought to be produced by activation of both postsynaptic dopamine D1 and D2 receptors [5-8]. In the rat nigrostriatal system, ascorbate is released, at least in part, by dopaminergic mechanisms, which appear to involve both the D1 and D2 family of dopamine receptors [3]. In many respects, ascorbate appears to function like a dopamine receptor antagonist. For example, pretreatment with ascorbate (500-2000 mg/kg) has been found to block amphetamine-induced focused stereotypy [9] and locomotion [10] in mice. In addition, ascorbate has been reported to potentiate both the cataleptic and anti-amphetamine effects of haloperidol [11], and similar results have been obtained with direct intrastriatal applications of ascorbate [12]. Other studies have also shown that ascorbate pretreatment reduces ipsilateral turning behavior produced by amphetamine in rats with unilateral lesions of the nigrostriatal dopaminergic pathway [13]. Recent studies had shown Dopamine...
receptors activities are moderated by ascorbic acid. Application ability of these studies in dopamine receptor function has been reported. As the behavioral evidence of anti-dopaminergic effects of ascorbic acid protection [3-6, 8]. Results of these studies suggest that ascorbate should be able to attenuate the stereotyped licking behavior induced by the mixed dopamine D1/D2 receptor agonist; apomorphine in rat. In this report, we assessed the effects of acute treatment with ascorbate alone or combined with the dopamine D1 receptor antagonist SCH 23390 or the dopamine D2 receptor antagonist sulpiride on apomorphine induced licking behavior in rats.

MATERIALS AND METHODS

Animals
All experiments were carried out on male Sprague-Dawley rats from the Pasteur Institute (Iran), 200-250 g body weight. The animals were housed 5 per plastic cage in an animal room maintained at 21 ± 2°C on a 12-h light/dark cycle (lights on 0700-1900 h). Standard laboratory rat chow (Pars, Iran) and water were available at all times except during the experiments. Each animal was used once only.

Chemicals
The following drugs were used: R (-)-apomorphine HCl (Research Biochemicals, USA), ascorbic acid (Merck, Germany), SCH 23390 (Research Biochemical, USA) and sulpiride (Sigma, USA). Ascorbic acid solutions were prepared in saline and the pH was adjusted to 7.2 ± 0.1 with sodium hydroxide. Other drugs were also dissolved in saline, except for sulpiride which was dissolved in a drop of acetic acid and then diluted with saline. The vehicle control was acetic acid in saline. Drug concentrations were prepared so that the necessary dose could be injected in a volume of 1 ml/kg i.p. or s.c..

Licking measurement
The rats were placed individually in a glass cylinder (25 cm wide, 25 cm high) and a mirror was arranged in an oblique position under the cylinder to make recording of licking possible. The animals were allowed 30 min to accommodate prior to testing. Immediately after apomorphine administration, the animals were put into the cylinder and the number of licks (protrusion of the tongue against the cylinder wall or floor) was recorded with a hand counter during a 75-min period. The experimental protocol was approved by the Research and Ethics Committee of Mazandaran University of Medical Sciences.

STATISTICAL ANALYSIS
One-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparisons test was used for statistical analysis. Differences with P < 0.05 between experimental groups at each point were considered statistically significant. All data were analyzed with the computer program, GRAPHPAD software (V2.01).

RESULTS

Licking behavior induced by apomorphine
Subcutaneous injection of different doses of apomorphine (0.125, 0.25, 0.5, 0.75, 1, and 1.25 mg/kg) to rats induced dose-dependent licking behavior [F (6-44) = 59.082, P < 0.0001] (Fig. 4-1). The maximum response was obtained with 1 mg/kg of the drug. The dose of 0.5 mg/kg of apomorphine was chosen for the induction of licking behavior in subsequent experiments because the ED50 value equaled 0.545 mg/kg (obtained by regression analysis).

Effects of ascorbic acid alone
In Fig. 4-2, it can be seen that pretreatment of the animals with ascorbic acid (200-350 mg/kg, s.c., 30 min before apomorphine) significantly decreased the licking behavior [F (3, 34) = 10.815, P < 0.0001, n=7-9 rats/group]. The ascorbic acid response was dose-dependent, with an ED50 of 229 mg/kg. Therefore, the dose of 250 mg/kg (ED61) was chosen for the subsequent experiments.

Ascorbic acid-SCH 23390 combinations
Pretreatment of animals with 250 mg/kg, s.c., ascorbic acid potentate the inhibitory effect of 0.5 and 1 mg/kg, i.p. SCH 23390 [F (5, 43) = 78.147, P < 0.0001, n=8-9 rats/group] (Fig. 4-3). SCH 23390 (0.5 and 1 mg/kg, i.p) when administered 30 mins before apomorphine, significantly decreased the licking behavior. No significant differences were found between two doses of 0.5 and 1 mg/kg, SCH 23390 in reducing of the licking behavior. Compared to these doses of SCH 23390 alone, the ascorbic acid-SCH 23390 combinations significantly decreased the licking behavior (P < 0.01 and P < 0.05, respectively).

Ascorbic acid-sulpiride combinations
Pretreatment of animals with 250 mg/kg, s.c.,
ascorbic acid potentate the inhibitory effect of 0.5 and 1 mg/kg, i.p. SCH 23390 [F (5, 43) = 78.147, P < 0.0001, n=8-9 rats/group] (Fig. 4-4). SCH 23390 (0.5 and 1 mg/kg, i.p) when administered 30 mins before apomorphine, significantly decreased the licking behavior. No significant differences were found between two doses of 0.5 and 1 mg/kg, SCH 23390 in reducing of the licking behavior. Compared to these doses of SCH 23390 alone, the ascorbic acid-SCH 23390 combinations significantly decreased the licking behavior (P < 0.01 and P < 0.05, respectively)

![Figure 1](image.png)

**Figure 1**

Licking behavior induced by subcutaneous injection of different doses of apomorphine (0.125-1.25 mg/kg). Licking response was recorded for a 75-min period. Results are expressed as means + S.E.M. (n=7-9 rats/group). *P < 0.05, **P <0.001, different from control group.

![Figure 2](image.png)

**Figure 2**

Effect of ascorbate on apomorphine-induced licking behavior in rats. Animals were injected with ascorbate (200-350 mg/kg, s.c., 30 min before apomorphine) and saline (1 ml/kg, s.c., 30 min before apomorphine). Results are expressed as means + S.E.M. (n=7-9 rats/group). *P < 0.001, different from control groups.
DISCUSSION

In the present basic study, the effects of ascorbate on apomorphine-induced licking behavior in rats were examined. The main findings are as follows;

a) Ascorbate was remarkably effective in reducing of apomorphine-induced licking behavior.

b) Coadministration of SCH 23390 (0.5 and 1 mg/kg) plus ascorbate produced a higher decrease in the licking behavior than administration of SCH 23390 alone.

c) Pretreatment of animals with ascorbate, failed to potentiate the inhibitory effects of 25 and 50 mg/kg sulpiride.

The results of the present study suggest a dopamine D2 receptor antagonistic mechanism for ascorbate in reducing of apomorphine-induced licking behavior. At 25 and 50 mg/kg sulpiride, the licking behavior was sufficiently impaired such that ascorbate supplementation had no further effect. In other word, when dopamine D2 receptors were blocked by sulpiride, ascorbate failed to decrease the licking behavior. Unlike this result, ascorbate, which had no significant effect on the licking behavior when given with sulpiride, clearly potentiated the inhibitory effect of SCH 23390, even at the maximum response. Thus, it seems likely that ascorbate reduces the licking response via a dopamine D2 receptor antagonistic mechanism, as when dopamine D1 receptors were blocked by SCH 23390, apomorphine would stimulate dopamine D2 receptors. The anti-dopaminergic effect of ascorbate on striatal function was reported by several investigators [14, 15; 3]. Ascorbate has been shown to interfere with [3H]-spiperon binding in corpus striatum [16] and to antagonize and potentiate the behavioral effects of amphetamine [9] and haloperidol [17; 11], respectively. This vitamin has been found to block amphetamine-induced focused stereotypy [9] and locomotor activity [10] in mice. It has also been reported that ascorbate potentiates the ability of haloperidol to block amphetamine-induced behaviors in rats and also potentiates haloperidol-induced catalepsy, a model of dopamine receptor blockade in the neostriatum of rats [11]. These results point to ascorbate as an antagonist of dopaminergic function in the neostriatum. A considerable amount of evidence indicates that ascorbate acts directly at dopamine receptors. Analysis of dopamine D1 or D2 receptor inhibition by ascorbate demonstrated that ascorbate alters dopamine receptors function either as an allosteric inhibitor or as an inducer of iron-dependent lipid peroxidation [3, 13]. It also is possible that ascorbate may influence dopamine transmission indirectly by acting on other neurotransmitter systems that, in turn, modulate the dopaminergic system. This interpretation is supported by some evidence showing that ascorbate can increase glutamatergic transmission [4], and glutamate has
also been described as having opposed effects to dopamine in the neostriatal function [18]. In fact, ascorbate is released from glutamatergic neurons as part of the glutamate reuptake process, in which the high-affinity glutamate transporter exchanges ascorbate for glutamate [3, 19; 20]. This process is unaffected by glutamate receptor antagonists but blocked completely by drugs known to prevent the operation of the glutamate transporter [20]. Ascorbate release is also regulated, at least in part, by dopaminergic mechanisms, which appear to involve both the dopamine D1 and D2 receptors [3]. The postsynaptic action of dopamine in the neostriatum is also regulated, at least in part, by glutamatergic mechanism [21; 22] Glutamate appears to oppose the effects of dopamine on neostriatal function [18; 23; 24], and to extent that ascorbate can potentiate the action of glutamate. Therefore, it is possible that an ascorbate-induced increase in striatal glutamate transmission underlies, at least in part, the ability of ascorbate to antagonize the licking behavior induced by apomorphine. [13], these results suggest that the inhibitory effect of ascorbic acid on amphetamine-induced licking behavior is mediated by dopamine D2 receptor mechanisms.

AUTHORS CONTRIBUTIONS

DF was the main investigator. HK, HRH were contributed to the study design and writing process. All authors read and approved the final manuscript.

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REFERENCES


7 Tolbert L.C, Morris P.E, Spollen J.J, Ashe S.C. Sterespecific effects of ascorbic acid


