Ascorbic acid decreases morphine self-administration and withdrawal symptoms in rats

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Abstract

Recent studies have indicated that the glutamatergic system is involved in the motivational aspects during the initiation of drug self-administration. Ascorbic acid (AA), an antioxidant vitamin, is released from glutamatergic neurons, and it modulates the synaptic action of dopamine and glutamate. In this study the AA effects on the self-administration of morphine and on the morphine withdrawal syndrome have been investigated.

Wistar rats were allowed to self-administer morphine (1 mg/infusion) during 10 consecutive days for 2 h/session. The number of lever pressings was recorded. An intraperitoneal AA injection (500 mg/kg, i.p.), 30 min before morphine self-administration produced a significant decrease in the initiation of morphine self-administration during all sessions. After the last test session morphine withdrawal symptoms (MWS) were recorded after naloxone precipitation. Most of MWS (but not all) were decreased by AA application. In conclusion, AA may change the motivational processes underlying the morphine self-administration.

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1. Introduction

Both acute and chronic administration of morphine affects the turnover of the classical brain monoamine neurotransmitters dopamine (DA) noradrenaline and 5-hydroxytryptamine [1,2]. Recent studies have indicated that the glutamatergic system is also involved in the morphine tolerance and abstinence [3–6]. Administration of glutamergic antagonist such as MK-801 and APV modulate the reinforcing effects of drug abuse in animals. Several studies have documented that MK-801 inhibits the cocaine self-administration [7] and reverses its facilitation after chronic exposure to amphetamine [8]. APV increases the lever pressings for cocaine when administered into the nucleus accumbens (NAC) in rats [9]. There is, however, little information about the effects of glutamate receptor antagonists on morphine self-administration.

Ascorbic acid (AA) is an antioxidant vitamin that is released from glutamatergic neurons as a part of the glutamate reuptake process, in which the high-affinity glutamate transporter exchanges AA for glutamate [10]. Although neurons are known to use this vitamin in many different chemical and enzymatic reactions, only recently some evidence has suggested a role for ascorbate in interneuronal communication. Experimental evidence suggests that AA plays a key role in modulating central glutamatergic [11] and dopaminergic [12] systems as well as behavior [13].

The perfusion of cerebral cortical neurons with high doses of AA (1–3 mM) rapidly and reversibly attenuates NMDA antagonist-induced inward currents [14]. Other experiments have shown that low doses of AA could increase the neuronal activity and induce widespread neuronal oxidative stress on the primary cortical neurons [15]. One possible explanation of the divergent effects of increasing AA concentration on neuronal activity is that AA may shift from a presynaptic facilitator of glutamate transmission at relatively low extracellular concentration to a blocker of postsynaptic glutamate

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receptors [16]. It has been reported that high doses of AA suppress the withdrawal symptoms in the opiate addicts [17] and prevent the development of tolerance and physical dependence on morphine in the mice [18,19]. Recently, it has been reported that exogenous AA markedly reduces the locomotor activity and the behavioral withdrawal responses in morphine-treated guinea pigs [20]. Since both glutamatergic and dopaminergic systems are involved in morphine tolerance and morphine withdrawal symptoms [21] and these behaviors are greatly affected by AA [16] it is interesting to evaluate the effects of AA on i.v. self-administration of morphine as well as on morphine withdrawal signs in rats.

2. Material and methods

2.1. Animals and housing conditions

Male wistar rats (250–300 g) were used. Before surgery for the infusion system on the basis of ethic committee permission of Isfahan University, the animals were group-housed and they received food and water ad libitum. They were maintained under a day–night cycle with lights on between 07:00 and 19:00 h. After surgery the animals were placed in individual home cages, and they were allowed to recover from the operation for 5 days before study itself was started. During the tests food and water were available ad libitum in the home cages. The day–night cycle was reversed for 3 days before tests, and the animals were recorded during the dark phase of the cycle.

2.2. Experimental design

(A) Male rats (n = 30) were divided into three groups:

1. Control group, which received saline in the self-administration sessions.
2. Morphine group, which received morphine in saline solution (concentration 5 mg/ml) during the self-administration sessions.
3. Ascorbic acid–morphine group (AA + morphine), which received both AA (500 mg/kg, i.p.) 30 min before each session and morphine in the self-administration sessions.

(B) To determine the effects of the acute administration of AA on morphine withdrawal symptom signs 40 morphine treated male rats (250–300 g) were used. They were divided into four groups:

1. Control group, which had access to tap water during 21 days.
2. Morphine group, which received morphine for 21 days.
3. Ascorbic acid group, which received morphine for 21 days, and then divided to three sub groups which received AA with three doses (100, 500 or 1000 mg/kg in saline i.p.) at the end of experiment.

2.3. Drugs

Morphine (HCl) was obtained from TEMAD Ltd., Teheran, Iran. Naloxone hydrochloride was purchased from Sigma Co., St Louis, USA. Ascorbic acid was purchased from Merck AG, Darmstadt, Germany. Morphine and AA were dissolved in saline.

2.4. Surgery

Animals were anaesthetized with ketamine (150 mg/kg) and rampon (0.1 mg/kg) and a cannula was inserted into the jugular vein. The cannula was guided subcutaneously up to the skull where it was fixed to a cured metal tube, which was secured on to the skull with small screws and fixed with dental acrylic cement.

2.5. Procedure

Details of the procedure have been reported previously [22]. Briefly, testing was done in standard conditioning cage (21 cm × 21 cm × 28 cm) placed in a sound attenuated room. The test cage was equipped with active and passive levers, 2 cm above the floor, and a red light located 4 cm above the active lever. The i.v. cannula of the animals tested was connected to the infusion pump. The pressing of the active lever, marked by the red light resulted in an i.v. infusion of 0.2 ml fluid (5 mg/ml morphine in saline solution or saline only) during 10 s. A depression of the active lever during this time (10 s) did not affect on the infusion of drug. The pressing of the passive lever had no programmed consequences.

The drug naïve animals were placed in the test cage and were allowed to i.v. self-administer a drug solution for 2 h a day (for 10 days). The number of the active and passive lever pressings is recorded by a oscillograph (Harvard).

2.6. Withdrawal syndrome signs

Withdrawal syndrome signs were precipitated with a s.c. naloxone injection (2 mg/kg). Withdrawal signs (body shakes, jumping, climbing, writhing, teeth chattering, irritability, ejaculation, ptosis and diarrhea) within 30 min after the injection of naloxone on the last day of experiment were recorded.
2.7. Statistical analysis

The data have been presented as means ± S.E.M. The data obtained during self-administration sessions were analyzed by using analysis of variance (ANOVA) with repeated measures (MANOVA). The number of active and passive lever pressings during every session were calculated. The differences in the total number of active and passive lever pressings (summed over ten sessions) between AA and morphine groups were analyzed by using Student’s t-test. This method of analysis also was used in the analysis of withdrawal signs.

3. Results

3.1. Initiation of drug self-administration

The self-infusion (SI) data during the initiation of morphine self-administration are presented in Fig. 1. Maximum values were obtained in a week. There was significant difference (ANOVA, \( P < 0.05 \)) between numbers of self-infusions in three groups. Self-infusion of the morphine alone resulted in an addiction of the rats as shown by the several fold higher pressing number of the active lever providing the morphine solution. There was no significant difference between control (saline) and morphine + AA groups, indicating that AA (500 mg/kg, i.p.) resulted in a significant decrease in the number of self-infusions (Figs. 1 and 2). The total number of active lever pressings in the AA receiving group was significantly less (t-test, \( P < 0.01 \)) than in the group receiving morphine only (Fig. 2). There was no significant difference between the total active lever pressings of the control and AA groups. The total number of the passive lever pressings in the AA, vehicle and morphine groups did not reveal any significant differences (ANOV A, \( P > 0.05 \)) (Fig. 3).

3.2. Withdrawal syndrome signs

Table 1 shows the effects of AA (500 mg/kg, i.p.) on the withdrawal syndrome signs. Controls did not show any of defined withdrawal signs. The i.p. administration of AA during the 10 days greatly attenuated most of the withdrawal signs specially ptosis, teeth chattering, wet dog shaking, jumping and climbing in comparison to these signs in the morphine group. In comparison with the morphine group, an acute AA administration 30 min before the naloxone injection greatly attenuated in morphine addicted rats the withdrawal signs like ptosis, teeth chattering and wet dog shaking, but it did not affect jumping, diarrhea, and it even increased ejaculations.

4. Discussion

In the present study AA injection (500 mg/kg, i.p.) 30 min before every session caused a decrease in the number of active
Table 1

<table>
<thead>
<tr>
<th>Withdrawal signs</th>
<th>Morphine group only</th>
<th>AA and morphine (100 mg/kg)</th>
<th>AA and morphine (500 mg/kg)</th>
<th>AA and morphine (1000 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet dog shakes</td>
<td>6.6</td>
<td>4.6</td>
<td>2.6*</td>
<td>1.6*</td>
</tr>
<tr>
<td>Teeth chattering</td>
<td>6.6</td>
<td>3.6*</td>
<td>1.6*</td>
<td>0.6*</td>
</tr>
<tr>
<td>Ptosis</td>
<td>6.6</td>
<td>1.6*</td>
<td>1.6*</td>
<td>0.6*</td>
</tr>
<tr>
<td>Jumping</td>
<td>6.6</td>
<td>5.6</td>
<td>6.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Climbing</td>
<td>6.6</td>
<td>6.6</td>
<td>5.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6.6</td>
<td>5.6</td>
<td>5.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Ejaculation</td>
<td>6.6</td>
<td>3.6*</td>
<td>5.6*</td>
<td>6.6*</td>
</tr>
<tr>
<td>Salivation</td>
<td>6.6</td>
<td>2.6*</td>
<td>0.6*</td>
<td>0.6*</td>
</tr>
<tr>
<td>Lacrimation</td>
<td>6.6</td>
<td>3.6*</td>
<td>0.6</td>
<td>0.6*</td>
</tr>
</tbody>
</table>

Numbers denote the number of rats showing positive signs relative to the total number of rats tested.

* P < 0.05 compared with the morphine withdrawal group by the Fisher exact test.

Numbers were depressed in AA receiving rats in comparison with the morphine addicted animals. These findings are in agreement with the previous studies, which have shown that AA suppresses MWS in opiate addicts [23] and prevents the development of tolerance to and physical dependence on morphine in mice [24] and reduces the locomotor and behavioral withdrawal responses in morphine treated guinea pigs [20]. More recently Evangelou and Kalfakakou [23] have shown that a high orally administered AA dose decreased the withdrawal syndrome problems in heroin addicts.

We also found that, compared to the morphine group, the AA administration 30 min before each session significantly decreased, most of the withdrawal signs after naloxan injection. These results showing the inhibitory effects of chronic AA administration on opiate withdrawal signs confirm previous findings in humans [25] and mice [24].

On the other hand the injection of AA (500 mg/kg, i.p.) 30 min before naloxone injection in morphine addicted rats (acute effect) had no significant effects on some of morphine withdrawal signs including: jumping, climbing and diarrhea, and even it could increase ejaculation, although it could significantly reduce the head/body shaking and face washing and rearing (Table 1). These findings suggest that they are controlled by different mechanisms. It may also reflect a poor penetration of AA into the brain that has been reported following acute systemic AA administration [26]. Apart from these observations rats exhibited no other significant changes in the spontaneous behavior following acute or chronic administration of AA.

The mechanisms involved in the suppression of the tolerance development to and physical dependence on morphine and morphine withdrawal response by chronic AA treatment is unknown. That could include an inhibition of opioid receptor binding by AA, effects on second messengers or more subtle effects on transmitter release and neuronal function [27]. Although the inhibition of opioid receptor binding by AA has been observed in guinea pig brain homogenates, this effect is unlikely to have been involved in the present study, because AA has no effect on opioid receptor binding in intact tissue preparations [28]. Likewise, although AA has been shown to suppress the opiate induced compensatory increase of cAMP in NG 108-15 hybrid tumor cells [29], the significance of this change in cAMP with respect to development of opiate dependence in normal brain has been questioned [30].

An intriguing possibility arises from the reported finding that AA and morphine have opposite effects on membrane Na⁺,K⁺-ATPase activity. AA has an inhibitory effect on this pump whereas morphine has a stimulatory effect [31,32]. Increased Na⁺, K⁺-ATPase activity has been suggested to be at least partly responsible for the two major opiate effects in the brain viz. membrane hyperpolarization and inhibition of neurotransmitter release [33]. Chronic AA administration may decrease the stimulatory effect of morphine on this pump activity and inhibit the development of dependence and thus the subsequent withdrawal response. As AA is a powerful hydrophilic antioxidant high doses may also have a role on the redox potentials in the brain, although rat is able to synthesize...
its own AA. Some of the membrane proteins may be redox sensitive. In conclusion the present study has confirmed that chronic administration of AA inhibits the development of morphine dependence, and it inhibits the morphine withdrawal responses. Rat model seems to be useful in further studies, which are needed in the further clarification of the molecular mechanisms.

References


