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The effect of vitamin C on morphine self-administration in rats

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Abstract

Background:

Recent studies have shown that addiction may be caused by abnormality of neurotransmission in the brain. Two neurotransmitters that involve into morphine addiction are dopamine and glutamate. The glutamatergic and dopaminergic systems are also involved in morphine tolerance and morphine withdrawal syndrome signs. Ascorbic acid (AA), as the antioxidant releases from the glutamatergic neurons, modulates the action of the dopamine and glutamate systems. In this study, the effect of AA on morphine self-administration and morphine withdrawal symptoms has been investigated.

Materials and Methods:

Male Wistar rats (250 - 300g) were anesthetized with ketamine (11%) and xailazine (15%). The cannula was inserted into the right jugular vein, and it was fixed subcutaneously on the skull. After surgery the animals were placed in individual home cages, and they were allowed to recover from the operation for five days, before the test. The animals were subjected to self-administration morphine for12 consecutive days, two-hour/sessions. The number of infusions and number of active and passive lever pressings were recorded.

Results:

An intra peritoneal injection of Ascorbic acid (AA) (400 mg/kg, i.p.), 30 minutes before morphine self-administration, produced a significant decrease in 12 days self-administration of morphine and withdrawal syndrome signs ($P < 0.05$). The morphine withdrawal signs (MWS) were recorded after naloxone precipitation, which decreased significantly with the injection of AA (400,700mg/kg), (<0.05). The number of self-infusions and the number of active lever pressings had significantly decreased after AA injection (*P*

< 0.05).

Conclusion:

The chronic administration of AA may prevent the development of tolerance and physical dependence on morphine self-administration via the glutamatergic system.

Keywords: Ascorbic acid, morphine, rat, self-administration

INTRODUCTION

Both acute and chronic administration of morphine affects the turnover of the classical brain neurotransmitter, dopamine. On the other hand, the glutamatergic system is also involved in morphine tolerance and abstinence.[1,2] It has been shown that the reinforcing effects of drug abuse are modulated by the administration of morphine, in animals.[3] Several studies have documented that MK-801(antagonist glutamate) inhibits cocaine self-administration in animals[4] and reverses its facilitation after chronic exposure to amphetamine.[5] AP5 (antagonist glutamate) increases the lever pressings for cocaine when administered into the nucleus accumbens (NAC).[6] Ascorbic acid is an antioxidant vitamin that has been an antagonist in the glutamatergic and dopaminergic systems.[7] A recent study has indicated that AA is released from glutamatergic neurons as a part of the glutamate reuptake process, in which the high-affinity glutamate transporter exchanges AA for glutamate.[8] AA plays an important role in the modulating action of the glutamatergic[6] and dopaminergic[6] systems, as well as in the behavior.[6] Low doses of AA can increase the neuronal activity and induce public neuronal oxidative stress on the primary cortical neurons.[9] The divergent effects of the overdoses of AA on the neuronal activity provide a change from the presynaptic facilitator of glutamate transmission, at relatively low extracellular concentration, to the discontinuation of postsynaptic glutamate receptors.[6] The high dose of AA also decreases the withdrawal symptoms in the opiate addicts,[10] changes the motivational process underlying morphine self-administration, and prevents the development of tolerance and physical dependence on morphine, in mice.[11,12] Both the glutamatergic and dopaminergic systems are involved in the dependence and tolerance of morphine and morphine withdrawal syndrome signs,[13] and all these treatments are majorly affected by AA.[14,15] From the previous studies it is not clear as to what dose of AA can prevent tolerance to opioids (especially morphine). Therefore, in this investigation we endeavor to determine the effect of low and high doses of AA on self-administration of morphine as well as on morphine withdrawal symptoms, in rats.

MATERIALS AND METHODS

Drugs

Morphine (HCl) was obtained from TEMD Ltd. Tehran, Iran.

Ascorbic acid was purchased from Merck, Germany. Morphine and ascorbic acid were dissolved in saline. Naloxone hydrochloride was obtained from Jaber EbnHaian, Tehran, Iran.

Experimental design

The male Wistar rats (n = 30) were divided randomly into three groups as follows:

- Control group (n = 10), received normal saline in the self-administration, for 12 consecutive days, two-hour/session
- Morphine group (n = 10) received morphine (HCl) in saline solution (5mg/ml) during the self-

administration for 12 consecutive days, two-hour/session

- Ascorbic acid + morphine group (n = 10) received both AA (400mg/kg, i.p) 30 minutes before each session and morphine in the self-administration for 12 consecutive days, two-hour/session.

Animals and housing conditions

Male Wistar rats (250-300g) were housed individually and received food and water *ad libitum*. The animals were maintained under a day-night cycle with lights on between 08:00 and 20:00 hours. After surgery, the animals were returned to their individual home cages and allowed to recover from the operation for five days before the test. The day–night cycle was reversed for four days before thetests, and the experiments were done during the dark phase of the cycle.

Surgery

The rats were anaesthetized with ketamine (11%) and xailazine (15%). The cannula filled with heparin-saline was inserted into the right jugular vein and it was guided subcutaneously up to the skull. The cannula was fixed on the skull with metal screws and dental acrylic cement.

Procedure

The experiment was done in a standard metal cage (21cm*21cm*28 cm) placed in a sound attenuated room. The test metal cage had two levers (active and passive) that were 2 cm above the floor, and a red light located 4 cm above the active lever. The rat i.v cannula was connected to the infusion peristaltic pump and pressing of the active lever, marked by the red light, resulted in an i.v infusion of 0.1 ml fluid (5 mg/ml morphine (HCl) in saline) for 10-12 seconds. The pressing of the passive lever had no i.v infusion. The animals were allowed to self-administer morphine for 12 consecutive days, two hours/session. For all groups during the first five days, when the animals pressed to active lever, they received foods and saline (control groups) or morphine (morphine, morphine + AA groups), on the other days when the animals pressed to active lever, they received saline (control groups) or morphine (morphine, morphine + AA groups). Thenumber of lever pressings was recorded by the computer and analyzed by a special program.

Withdrawal syndrome signs

Acute and chronic effect of ascorbic acid Acute

Male Wistar rats (n = 24) were tested in four groups as follows:

- Control group (n = 6) received 0.2 ml normal saline for nine consecutive days
- Morphine group (n = 6) received morphine (10 mg/kg, 20 mg/kg, 40 mg/kg) for nine consecutive days; the first three days 10 mg/kg, the second three days 20 mg/kg, and the third three days 40 mg/kg[7]
- Morphine + AA group (n = 6) received morphine (10mg/kg, 20mg/kg, 40mg/kg) for nine consecutive days, and on the tenth day, they received ascorbic acid (400,700 mg/kg, i.p) for evaluating the effects of AA on the morphine withdrawal syndrome signs (MWS). At the end of the training period, all the animals were put into abox made of fiber glass, and the signs of withdrawal were controlled and measured for 30 minutes after injection of naloxone (2 mg/kg, i.p).

Chronic

The control and morphine groups are the same, with an acute effect of AA. In the groups three animals received 400 mg/kg AA before injection of morphine for nine consecutive days. The signs of withdrawal were measured for 30 minutes after injection of naloxone (2 mg/kg, i.p) on the tenth day.

Statistical analysis

Comparison between the two groups was performed using the unpaired Student's *t*-test. Comparisons among several groups were performed using one-way analysis of variance (ANOVA) and the *post-hoc* test (Turkey). A value of $P < 0.05$ was considered statistically significant.

RESULTS

Initiation of drug self-administration

Our results indicated that the number of active lever pressings in the three groups were significantly different within the groups [Figure 1, $P < 0.05$]. The total number of active lever pressings was increased between the morphine group and the control group. After administration of ascorbic acid, the number of active lever pressings started to decrease significantly from the fifth day until the twelfth day, when compared to the morphine group ($P < 0.05$).

There was no significant difference between the number of self-administrations in the three groups in the earliest days (the first, second and third days). After five days, a significant difference was observed among the number of self- administrations in the three groups [Figure 2, $P < 0.05$]. Self-administration in the AA + morphine group was significantly less than that in the morphine group ($P < 0.01$).

There was no significant difference among the number of passive lever pressings in the three groups during the 12 days [Figure 3, $P > 0.05$]. The total number of passive lever pressings also did not have significant changes between the morphine group and the other groups. The numbers of active and passive lever pressings were compared between each group of rats. These results indicated that the number of active lever pressings increased significantly in the morphine group ($P < 0.05$), but after injection of ascorbic acid decreased in the morphine + AA group [Figure 4].

Our results showed that intraperitoneal administration of AA (400 mg/kg, i.p) prevented the development of tolerance and dependence on morphine. Injection of ascorbate could antagonize the reinforcing effect of morphine and could be used as an effective pharmacotherapy for morphine abuse, especially when a high dose was used.

Withdrawal syndrome signs

Results from the effect of acute and chronic ascorbic acid on the abstinence syndrome are shown in Table 1 . In these results, the control group did not show any of the defined withdrawal signs. Most of the signs were reduced after acute injection of AA, especially teeth chattering, wet dog shakes, elongated body posture, and climbing, in comparison to these signs in the morphine group. Chronic administration of AA before injection of morphine for nine days greatly attenuated the withdrawal signs like climbing, teeth chattering, licks, and wet dog shaking, in morphine-addicted rats, but it had no effect on jumping or diarrhea.

DISCUSSION

In this study, the injection of AA (400 mg/kg, i.p) 30 minutes before the self-administration session caused a decrease in the number of active lever pressings to obtain morphine [Figure 1]. The tolerance in the groups had decreased significantly in rats receiving AA in comparison tothe morphine-addicted animals [Figure 1]. A previous study showed the effect of AA on morphine self-administration and morphine tolerance according to various doses.[6] The results indicated that 400 mg/kg of AA changed morphine withdrawal signs and morphine dependence, but 700 mg/kg of AA changed morphine tolerance without effect on the signs of active lever pressing, after morphine injection, during self-infusion [Figure 2, tab].

These results showed that various doses of AA produced different tolerance and withdrawal signs [Table 1]. We had determined that the animals would die if AA of more than of 700 mg/kg was used. This dose of AA, might enhance the activity of the dopaminergic system and create a problem for the animals and humans. This was a novel finding of this experiment. Evanelou and Kalfakakou,(2000)[16] showed that withdrawal syndrome signs decreased in heroin addicts, with oral administration of high doses of AA. These studies indicated that the inhibitory effects of chronic AA administration on morphine withdrawal symptoms confirmed the previous findings in humans and mice.[7,17]

The rat receiving repeated treatment with saline followed by various doses of morphine (10, 20, 40mg/kg) displayed numerous escape jumps in response to administration of noloxane (2 mg/kg, s.c.) on the tenth day. The rat treated with AA (400 mg/kg, i.p) followed by morphine for nine days showed a decrease in the incidence of escape jumps. However, this effect of AA was not significant.

It is possible that these symptoms were controlled by various mechanisms. It may also reflect a poor penetration of AA into the brain, which has been reported following acute systemic AA administration.[18] The process involves development of physical dependence on morphine, and suppression of tolerance and withdrawal response by chronic AA behavior is unknown.

Recent studies have reported that AA and morphine have opposite effects on membrane Na, K^+- ATPase activity. Morphine has a stimulatory effect, whereas, AA has an inhibitory effect on this pump Na^+, k^+-ATPase.[6,15] It has been reported that an increase in the activity of Na, K^+ ATPase is partly responsible for the two major opiate effects in the brain, which create hyper polarization in the membrane and also inhibition of neurotransmitter release.[14] The AA dose dependently decreases the analgesic effect of morphine in mice,[19] and it may reduce the analgesic effect of morphine in patients. These results, along with others' knowledge, show that AA modulates the synaptic action of dopamine and glutamate, which suggests that even very high doses of AA, which are known to be non-toxic, may be used with dopaminergic antagonist drugs, to treat the morphine-abstinence syndrome.

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Footnotes

Source of Support: Nil

Conflict of Interest: None declared.

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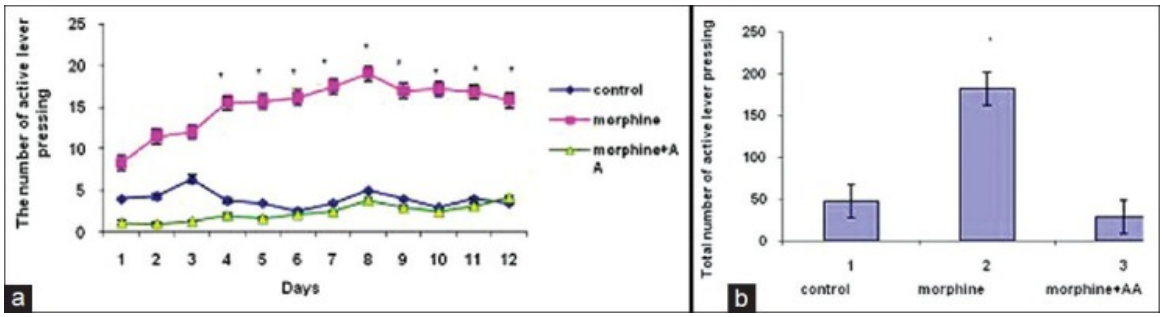
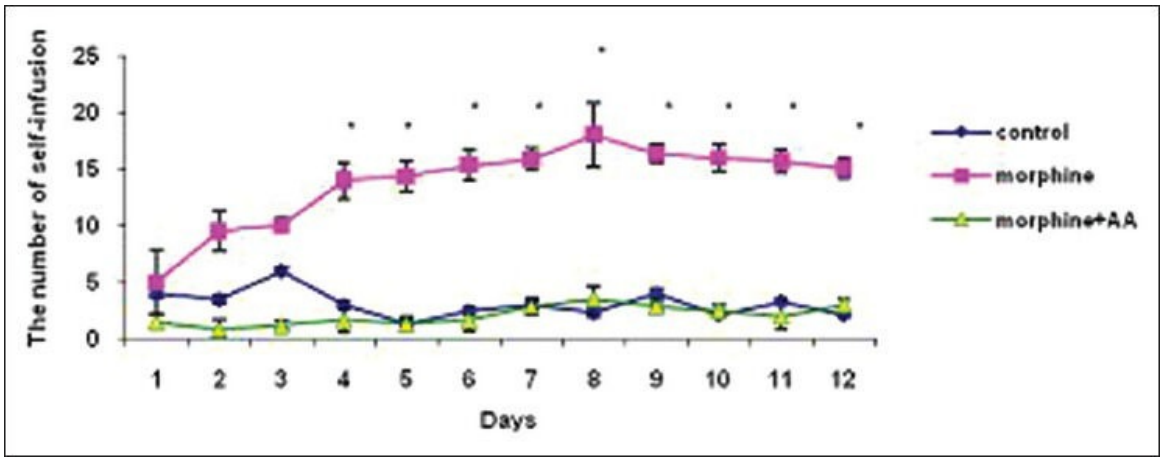
Figures and Tables

Figure 1



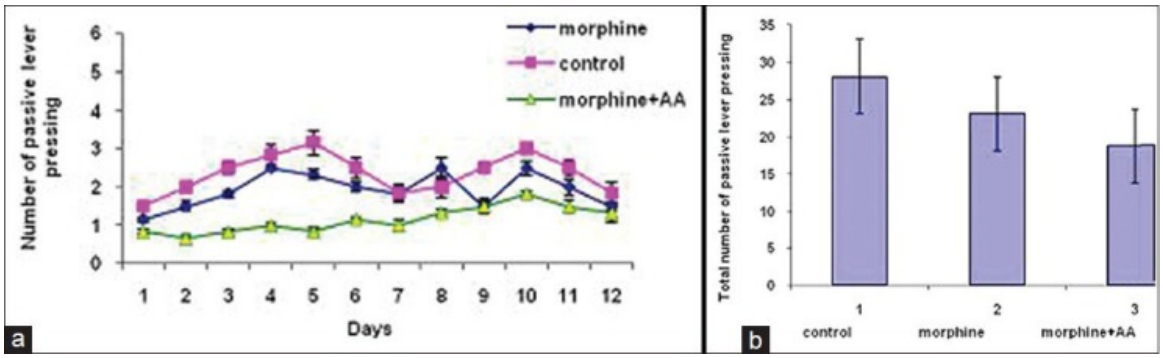
The number of active lever pressings in the three groups (control, morphine, morphine AA): (a) There are significant differences between the morphine group and the other groups during the 12 days. ($P < 0.05$, N = 10, X ± SEM). The number of lever pressings is decreased after injection of AA from the fifth to the twelfth days. (b) the total number of active lever pressings in the three groups of experiments: There is a significant difference between the morphine alone and the control and morphine + AA groups. (X ± SEM, $P < 0.05$). Confidence interval (CI) Morphine, morphine + AA: 9.407 < CI < 13.955 Morphine, control: 8.306 < CI < 12.971 Morphine + AA, control: -1.927 < CI < 0.152

Figure 2



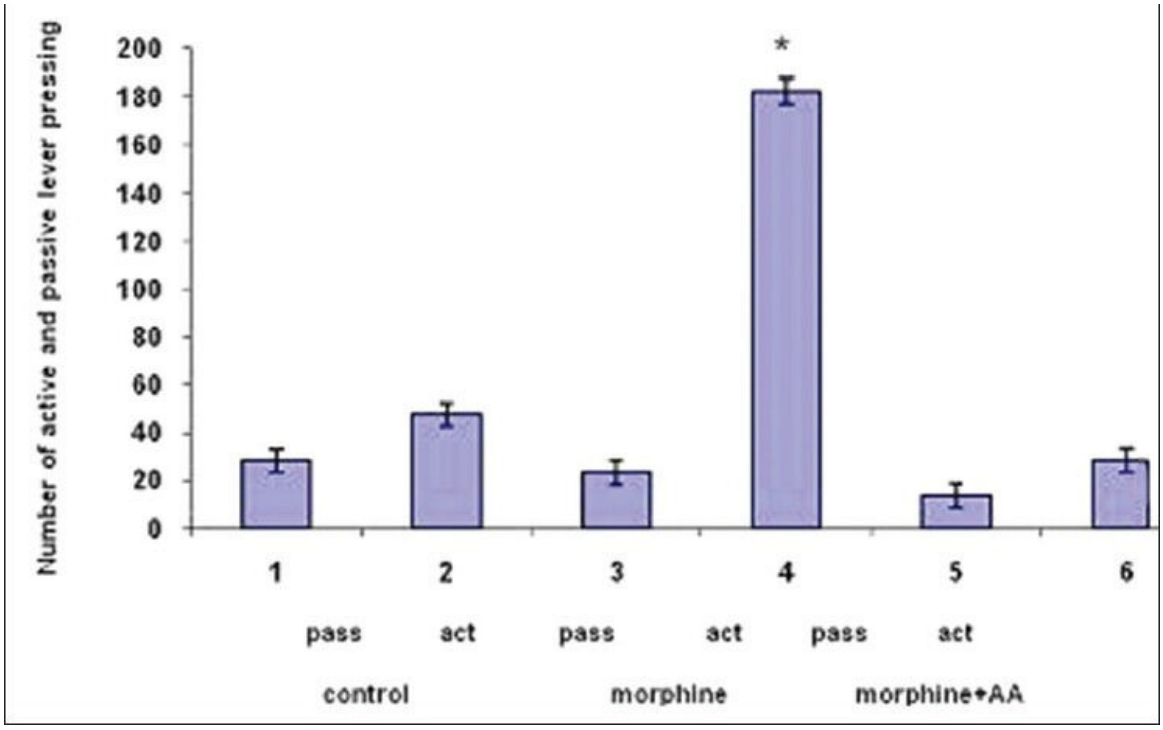
The number of self-administrations in three groups of experiments. There is no significant difference between the AA + morphine and control groups. The number of infusions in the AA + morphine group is decreased in comparison to the morphine group ($P < 0/05$, N = 10, X ± SEM)

Figure 3



The number of passive lever pressings in three groups of experiments:(a) There is no significant difference between morphine, AA + morphine, and the control groups during the 12-dayself-administration session. (N = 10, X ± SEM);(b) there is no significant difference between the morphine, control, and morphine + AA groups in the total number of passive lever pressings. ($P < 0.05$) X ± SEM. Confidence interval morphine, morphine + AA: -0.431 < CI < 1.126 Morphine, control: -0.829 < CI < 0.042 Morphine + AA, control: -1.571 < CI < 0.819

Figure 4



The total number of passive and active lever pressings was compared between each group. The active lever pressing was significantly higher than the passive only in the morphine group ($P < 0.05$). The total number of active lever pressing reduced after injection of AA and there was no significance with passive lever pressing in the morphine + AA group

Table 1

The effect of ascorbic acid on the morphine withdrawal signs during the 30 minutes after injection of naloxone

Withdrawal signs	Morphine group	Morphine+AA (400)	Morphine+AA (700)
Acute			
Climbing	7.6±0.8	4±0.36	3.6*±0.66
Teeth chattering	6.8±1.10	1*±0.36	0*±0
Lick	10±1.48	3.8*±0.70	0.6*±0.21
Elongated body posture	7.5±1.75	2.6*±0.55	1.8*±0.54
Shake in body	5.6±0.95	2.3*±0.49	0*±0
Aching	7.8±1.13	1.8*±0.3	0.1*±0.16
Diarrhea	0.33±0.3	0.1±0.22	0.3±0.21
Jumping	2±0.51	1.16±0.33	2±0.51
Chronic			
Climbing	7.6±0.8	3.66*±0.61	-
Teeth chattering	6.8±1.1	0*±0	-
Lick	10±1.48	1.33*±0.8	-
Elongated body posture	5.7±1.4	2.5*±0.5	-
Shake in body	5.6±0.95	0.16*±0.16	-
Aching	7.83±1.13	0.83*±0.3	-
Diarrhea	0.33±0.21	0.16±0.16	-
Jumping	2±0.51	1.66±0.42	-

Numbers denote the number of rats showing positive signs, relative to the number of rat experiments, *$P < 0.05$ comparative between morphine groups and morphine+AA groups. These signs were significantly decreased in morphine+AA (400) groups relative to morphine group. The number of mean±SEM for each group

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