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Pharmacology, Biochemistry and Behavior 77 (2004) 111–116
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Effects of nitric oxide on morphine self-administration in rat

Hedayat Sahraei[a,b], Gholamreza Poorheidari[c], Mohsen Foadaddini[d], Ali Khoshbaten[a,b], Alireza Asgari[a,b], Ali Noroozzadeh[a,b], Hassan Ghoshooni[a,b], Seyed Hassan Firoozabadi[a,b], Mohammad-Reza Zarrindast[e,f,*]

[a]*Department of Physiology, Baghyatallah (a.s.) University of Medical Sciences, Tehran, Iran*
[b]*Behavioral Science Research Center (BSRC), Baghyatallah (a.s.) University of Medical Sciences, Tehran, Iran*
[c]*Department of Pharmacology, Baghyatallah (a.s.) University of Medical Sciences, Tehran, Iran*
[d]*Department of Physiology, School of Medicine, Birjand University of Medical Sciences, Birjand, Khorasan, Iran*
[e]*Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[f]*Iranian National Center for Addiction Studies, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

Received 3 June 2003; received in revised form 24 September 2003; accepted 7 October 2003

Abstract

Previous studies have reported that morphine exerts its effects in part through the release of nitric oxide (NO). In the present study, the effects of acute and chronic administration of the NO precursor, L-arginine and NO synthase (NOS) inhibitor, L-nitro-amino-methyl-ester (L-NAME) on morphine self-administration in rats were investigated. The animals were initially trained to press a lever using food as reinforcer. Rats were surgically prepared with a chronic Silastic catheter implanted in the external jugular vein. Five days after surgery, they were trained to press a lever for drug self-administration. The present data indicate that L-arginine (0.05, 0.1, and 0.15 mg/kg/injection) but not L-NAME (0.05, 0.1, and 0.15 mg/kg/injection) induced self-administration behavior and increased locomotion. The response induced by L-arginine (0.1 mg/kg/injection) was reduced by pretreatment with L-NAME (5, 10, and 15 mg/kg ip). Both the acute (5, 10, and 15 mg/kg ip) and the chronic (200 mg/kg ip; twice daily for 4 days) administration of L-arginine reduced morphine self-administration. However, acute (5, 10, and 20 mg/kg ip) and chronic (50 mg/kg ip; twice daily for 4 days) administration of L-NAME increased morphine self-administration significantly. It can be concluded that NO may have a role in morphine self-administration.

Keywords: Morphine; Self-administration; Nitric oxide; L-Arginine; L-NAME

1. Introduction

The role of the mesolimbic dopaminergic system in opioid reward is well known (Spanagel and Welss, 1999). Several experiments have shown that dopamine may not be the only neurotransmitter responsible for opioid reward (Koob and Bloom, 1988; Pettit et al., 1984; Van Ree and Ramsey, 1987). Other studies have revealed that glutamate receptor antagonists block the development and expression of morphine reward (Layer et al., 1993; Tzschentke and Schmidt, 1997, 1998). This effect of glutamate is thought to be mediated in part by nitric oxide (NO) (Bredt and Snyder, 1992; Garthwaite et al., 1989). Furthermore, some data show that NO synthase (NOS) inhibitors can prevent the development of tolerance to morphine (Kolesnikov et al., 1992; Fairbanks and Wilcox, 1997; Xu et al., 1998) and morphine dependence (Kolesnikov et al., 1993). Morphine administration can also modulate the expression of NOS (Cuellar et al., 2000; Lysle and How, 1999). On the other hand, there is some evidence that NO may be involved in the rewarding properties of morphine (Kivastik et al., 1996; Biala and Langwinski, 1996). Recent studies indicate that NO activation and NOS inhibition in amygdala (Zarrindast et al., 2002), the CA1 area of hippocampus (Karami et al., 2002), and the nucleus accumbens (Gholami et al., 2002) may influence morphine-induced conditioned place preference in rats. The effect of acute and chronic administration of L-arginine on morphine self-administration has not been determined. In the present study we examined the effects of acute and chronic treatment with L-nitro-amino-methyl-ester (L-NAME; NOS inhibitor) and L-arginine (NO precursor) on

* Corresponding author. Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran. Tel.: +98-21-611-2801; fax: +98-21-460-2569.
E-mail address: zarinmr@ams.ac.ir (M.R. Zarrindast).

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doi:10.1016/j.pbb.2003.10.008

morphine self-administration in the rat to reveal the role of NO in morphine reward.

2. Materials and methods

2.1. Animals

Male Wistar rats (Pasture Institute, Tehran, Iran) weighing 250–350 g were housed five per cage in an animal room that was lit for 12 h/day (lights on at 7:00 p.m.). Food and water were available ad libitum. Eight animals were used in each experiment. The protocol has been approved by the Ethical Committee of Baghyatallah (a.s.) University of Medical Sciences, Research Department (206; September 12, 2000).

2.2. Surgical procedure

Animals were anesthetized by sodium pentobarbital (50 mg/kg ip) and catheters of silicone rubber (0.62 mm inner diameter, 1.0 mm outer diameter) were implanted into the right external jugular vein. The tip of the cannula was occluded with silicone adhesive (Silastic, Razi Chemical Iran) and a series of small holes were made near the tip with a sharp needle (Van Ree et al., 1978; Sahraei et al., 1999). This cannula prevents contact between blood and infusion fluids, until fluid pressure in the lumen of the cannula during infusion expands the tubing and opens the holes. The cannula was inserted into the vein where its tip was located near the right atrium. Its free end was guided subcutaneously to the skull and connected to polyethylene tubing by a U-shaped stainless steel tube that was cemented to the skull by dental acrylic cement and two screws. After surgery, animals were placed in individual cages and were allowed 5 days for recovery from the operation. All experiments were done in the dark phase of cycle.

2.3. Self-administration apparatus

Training and testing were done in standard operant conditioning cages placed in a sound-attenuated room, ventilated with fans, based on the method used previously by others (Van Ree et al., 1978) with minor modifications. The apparatus consisted of two chambers equipped with one lever that was marked by a red light placed just above the lever. The intravenous cannula of the animal was connected to an infusion pump via a swivel, allowing the animal to move relatively freely. Pressing of the lever resulted in a 15-s infusion of 0.1 ml fluid through an infusion pump. The red light went off during the infusion and further pressing of the lever during this time would not infuse further. In this study, the numbers of infusions are regarded as a measure of the reinforcing action of the drug. A series of LED infrared were mounted in the wall of the cage and the locomotor activity of the animals was counted during the experiments.

2.4. Self-administration procedure

The training procedure has been described in greater detail elsewhere (Hubner and Koob, 1990). Briefly, to aid in acquisition of drug self-administration, rats were initially trained to press a lever using food as a reinforcer before being surgically implanted with a chronic intravenous jugular catheter. Following 24 h of food restriction, rats were placed in the operant chambers where a lever filled with food pellets was available. Lever pressing resulted in the delivery of a 100-mg pellet on a fixed ratio (FR) 1 schedule. Each rat was allowed to self-train and press for 40 pellets before being returned to ad libitum food. Following acquisition of lever pressing behavior, rats were surgically prepared. Five days after surgery, the rats were trained to self-administer morphine (0.75 mg/kg/injection) for 2 h each day on an FR-1 schedule (Sahraei et al., 1999). Training days were between 8 and 10 days for morphine and 7 and 9 days for L-arginine. Between 9% of animals failed to self-administer the drugs. Changes of less than 10% in the number of injections in the last 3 days were recorded and used as baseline. Once the baseline of the drug self-administration was achieved, the experiments began. Catheters were flushed daily with saline (0.1 ml) during the recovery period as well as before and after the self-administration sessions. All operant sessions were conducted during the animals' dark cycle. Catheter patency was tested by injection of 0.1 ml solution of sodium pentobarbital (10 mg/ml) into the catheter and observation of animal behavior. Animals with patent catheters exhibit prominent signs of anesthesia (loss of muscle tone) few seconds after administration (Caine and Koob, 1994).

2.5. Measurement of locomotor activity

Locomotor activity was measured during the experiments by means of eight infrared LEDs mounted in the walls of the cage (four in the lateral wall 2 cm above the floor of the cage and four in the dorsal wall 2.5 cm above the floor of the cage where they cross each other).

2.6. Experimental design

2.6.1. Experiment 1: Effects of acute administration of L-arginine and L-NAME on self-administration and locomotion induced by morphine

To investigate the effects of L-arginine and L-NAME on the expression of morphine-induced self-administration and locomotor activity, once the baseline of morphine self-administration had been established, saline or various doses of L-arginine (5, 10, and 15 mg/kg ip) and L-NAME (5, 10, and 15 mg/kg ip) were injected 20 and 60 min before testing, respectively, in two different groups of animals (i.e., they receive only one injection through the experiments). The results have been shown in Fig. 1.

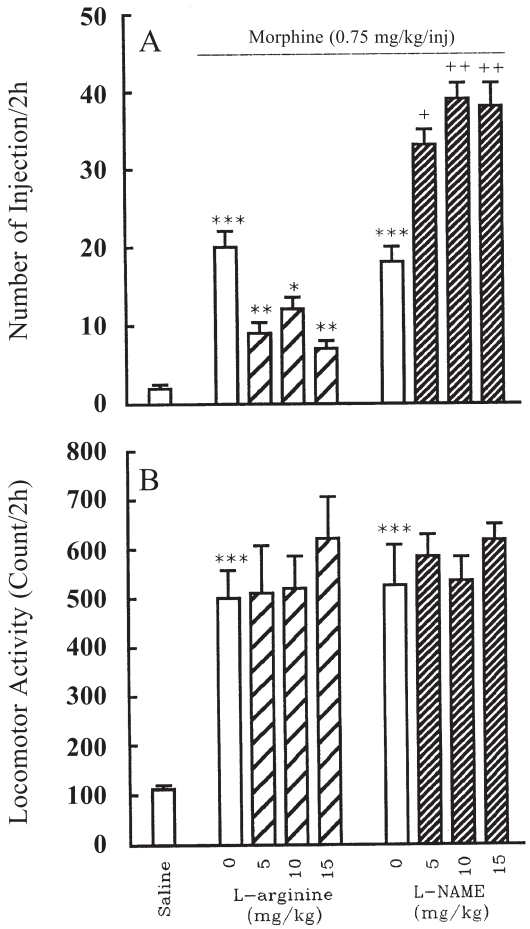

Fig. 1. Effects of acute administration of NO agents on morphine self-administration. Animals were injected with L-arginine (5, 10, and 15 mg/kg ip), 20 min, or L-NAME (5, 10, and 15 mg/kg ip), 60 min before the test and were given the opportunity to self-administer morphine (0.75 mg/kg/injection) for 2 h. Number of injections (A) and locomotor activity (B) were recorded (as described in Materials and Methods). Each point is the mean ± S.E.M. for eight rats. $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$, different from saline control group. $^{+}P<.05$, $^{++}P<.01$, different from respective control group.

2.6.2. Experiment 2: Effects of chronic treatment with L-arginine and L-NAME on morphine-induced self-administration and locomotor activity

To test the effects of chronic administration of L-arginine and L-NAME on morphine-induced self-administration and locomotion, once the baseline of morphine self-administration was established, saline, L-arginine (200 mg/kg ip; twice daily for 4 days; Bhargava et al., 1997) in the first group or L-NAME (50 mg/kg ip; twice daily for 4 days; Pulvirenti et al., 1996) in the second group of animals were injected intraperitoneally (last injections in each day were 2 h before testing). Thus, the animals received eight injections through the experimental sessions. Lower doses of the drugs did not alter morphine self-administration. The results have been shown in Fig. 2.

2.6.3. Experiment 3: Assessment of the self-administration and locomotor activity induced by NO-modulating drugs

L-Arginine and L-NAME were used on their own to examine whether they can induce self-administration (the procedure was similar to that used for morphine) and to test the effect of the drugs on locomotion. The animals were divided into 12 groups, which received saline (0.1 ml/injection), L-arginine (0.05, 0.1, and 0.15 mg/kg/injection), L-NAME (0.05, 0.1, and 0.15 mg/kg/injection), or L-NAME (5, 10, and 15 mg/kg ip) 60 min before L-arginine (0.1 mg/kg/injection) self-administration respectively. Self-administration and locomotor activity was recorded as described in the methods section. The results have been shown in Fig. 3.

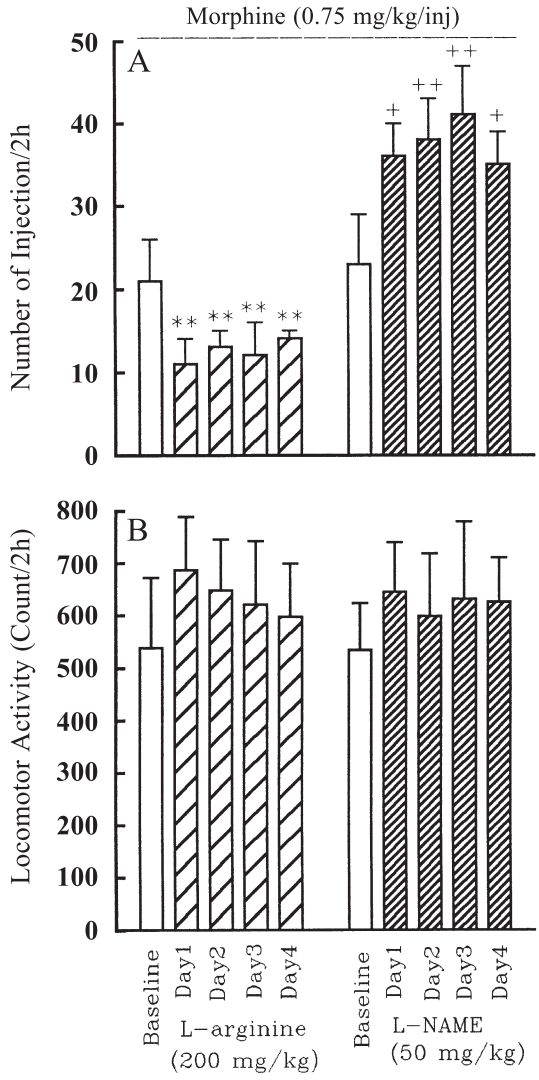

Fig. 2. Effects of chronic administration of NO agents on morphine self-administration by rats. Animals received L-arginine (200 mg/kg ip; twice daily for 4 days) or L-NAME (50 mg/kg ip; twice daily for 4 days) and then were given the opportunity to self-administer morphine (0.75 mg/kg/injection) for 2 h. Number of injections (A) and locomotor activity (B) were recorded (as described in Materials and Methods). Each point is the mean ± S.E.M. for eight rats. $^{**}P<.01$, $^{+}P<.05$, $^{++}P<.01$, different from respective control groups.

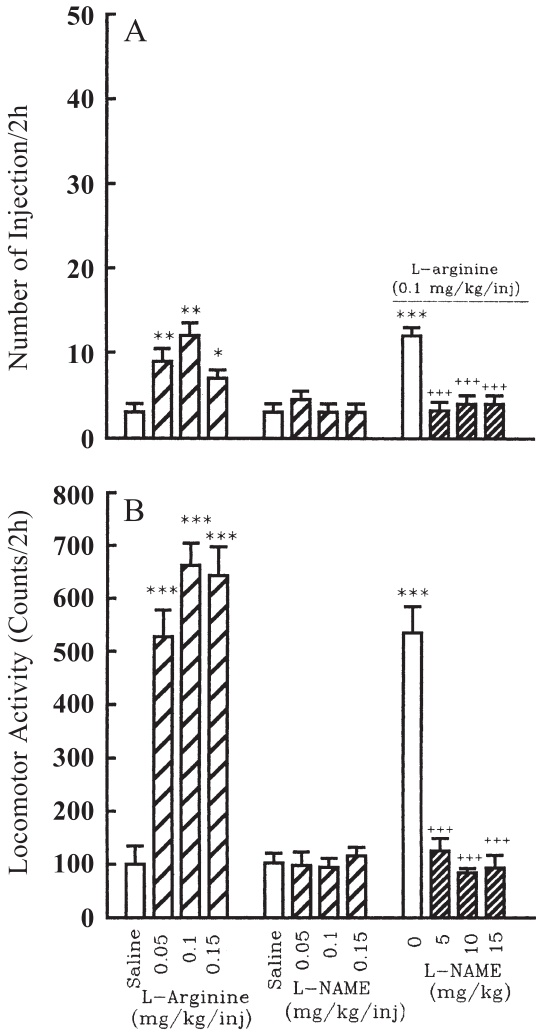

Fig. 3. Self-administration of L-arginine, L-NAME, or L-NAME plus L-arginine by rats. Animals were given the opportunity to self-administer different doses of L-arginine (0.05, 0.1, and 0.15 mg/kg/injection) or L-NAME (0.05, 0.1, and 0.15 mg/kg/injection), for a period of 2 h. L-NAME was given 60 min before L-arginine. Number of injections (A) and locomotor activity (B) were recorded (as described in Materials and Methods). Each point is the mean ± S.E.M. for eight rats. $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$, different from saline control group. $^{+++}P<.001$, different from L-arginine control group.

2.7. Drugs

The following drugs were used in these experiments: morphine sulfate (TEMAD, Iran), L-arginine, L-NAME, and sodium phenobarbital (Sigma, USA). All the drugs were dissolved in 0.9% saline. The drugs were given intraperitoneally in a volume of 1 ml/kg and were prepared immediately before use. The control groups received saline.

2.8. Data analysis

One-way analysis of variance (one-way ANOVA) followed by Tukey post *t* test was used to test statistical differences between groups. *P* values less than .05 were considered significant. All data are given as mean ± S.E.M.

3. Results

3.1. Effect of acute administration of L-arginine or L-NAME on morphine self-administration

Administration of L-arginine (5, 10, and 15 mg/kg ip) 20 min before the test decreased [$F(3,28)=5.0$, $P<.001$], while preadministration with L-NAME (5, 10, and 15 mg/kg ip) 60 min before the test increased morphine self-administration [$F(5,42)=9.3$, $P<.001$] (Fig. 1A). Morphine however, increased locomotion. One-way ANOVA showed that L-arginine [$F(3,28)=1.6$, $P>.05$] or L-NAME [$F(3,28)=1.2$, $P>.05$] did not alter the morphine response (Fig. 1B).

3.2. Effect of chronic administration of L-arginine or L-NAME on morphine self-administration

Chronic administration of L-arginine (200 mg/kg ip; twice daily for 4 days) decreased the morphine self-administration significantly [$F(4,35)=20.5$, $P<.0001$]. While chronic administration of L-NAME (50 mg/kg ip; twice daily for 4 days) increased the morphine self-administration significantly [$F(4,35)=8.7$, $P<.0001$] (Fig. 2A). One-way ANOVA indicated that L-arginine [$F(4,35)=1.6$, $P>.05$] or L-NAME [$F(4,35)=1.4$, $P>.05$] did not alter morphine-induced locomotor activity (Fig. 2B).

3.3. Self-administration behavior

Fig. 3 shows that when naïve rats were given the opportunity to self-administer saline, the number of self-administration were one to three injections. In contrast, in the animals that were given the opportunity to self-administer different doses of L-arginine (0.05, 0.1, and 0.15 mg/kg/injection) over 2 h, self-administration was achieved [$F(3,28)=13.4$, $P<.0001$]. Moreover, the different doses of L-NAME (0.05, 0.1, and 0.15 mg/kg/injection) did not induce self-administration in drug-naïve animals [$F(3,28)=1.18$, $P>.05$]. The response of L-arginine (0.1 mg/kg/injection) was decreased by L-NAME (5, 10, and 15 mg/kg ip) pretreatment [$F(3,28)=37.2$, $P<.0001$] (Fig. 3A). Moreover, L-arginine [$F(3,28)=33.4$, $P<.0001$] but not L-NAME [$F(3,28)=0.228$, $P=.876$] increased locomotion. The effect of L-arginine (0.1 mg/kg/injection) was decreased by L-NAME (5, 10, and 15 mg/kg ip) [$F(3,28)=51.7$, $P<.0001$] (Fig. 3B).

4. Discussion

There is limited information on the effect of NO on morphine self-administration. In the present study, attempts

were made to find out the effect of L-arginine, an NO precursor, and L-NAME, an NOS inhibitor, on morphine self-administration.

The present results show that L-arginine can induce self-administration (increased the number of lever pressing in rats) by itself. Since the drug increased locomotor activity, its influence on motor activity cannot be excluded. Similar to other drugs of abuse (Van Ree et al., 1978), L-arginine can produce an inverted U-shaped curve. However, self-administration behavior induced by L-arginine was dose-dependent. The response of the drug was decreased by L-NAME pretreatment. The data indicate that an increase in the NO levels by L-arginine may induce self-administration. It is also clear that NO is a powerful mediator for inhibiting dopamine transporters in the dopaminergic synapses that take up dopamine released from presynaptic neurons (Kiss, 2000; Pogun and Michael, 1994) and dopamine release (Silva et al., 1995; 2003). Furthermore, NOS immunoreactivity has been detected in the ventral tegmental area (Rodrigo et al., 1994), and over 30% of NOS is seen in vesicle-filled axons and axon terminals in the shell of the nucleus accumbens (Gracy and Pickel, 1997). These two sites are the main regions of the action of drugs of abuse in the brain (see Koob, 1992). Thus, increase in NO levels by L-arginine in the nucleus accumbens may decrease dopamine reuptake, thereby increasing the concentration of synaptic dopamine, which may account for the drug self-administration. Increased dopamine concentrations have been observed in all types of drugs of abuse (Hyman and Malenka, 2001; Pontieri et al., 1995).

The present data showed that L-NAME did not induce self-administration. The results may support the idea that inhibition of physiologic NO, has no influence on reward effects in rats. It has been also shown that the opioid receptor antagonist, naloxone does not induce self-administration (Van Ree et al., 1978). To study the effects of antagonists of abused drugs, it would be useful to apply other tests such as conditioned place preference (CCP).

In agreement with previous studies, our present data indicate that morphine could induce self-administration in rats (Van Ree et al., 1978; Sahraei et al., 1999). The present data indicate that acute and chronic administration of L-arginine decreased morphine self-administration in a dose-independent manner. While morphine self-administration was enhanced by acute and chronic injection of L-NAME, which can be observed by the increase in the number of lever pressing by rats. Since L-arginine elicits an increase of self-administration by itself, the possibility may exist that NO plays a modulatory role in morphine self-administration.

The effects of both drugs may be additive. This means that the reinforcing properties of L-arginine and morphine may be additive and thus may reduce self-administration behavior. In our study, it is possible that by the effect of NO derived from L-arginine to increase the concentration of dopamine in the synaptic area, the reinforcing effects of morphine are occluded. Furthermore, chronic injections of L-arginine had the same effects. Thus, increasing NO levels resulted in a reduction of morphine self-administration behavior. It is possible that an unknown pharmacokinetic interaction between morphine and L-arginine may have produced these results.

Since the effects of L-arginine and L-NAME in both the acute and chronic phases were dose-independent, it can be concluded that NO had no direct effect on morphine reward. It should be noted that the results of both acute and chronic administration of L-arginine and L-NAME on morphine self-administration appeared to be similar. It could be hypothesized that this is simply the result of the acute effects of NO-mediated substances, but it must be borne in mind that other investigators have also reported the similar results (Pulvirenti et al., 1996). However, it is difficult to explain why the acute and chronic actions of L-arginine and L-NAME are similar. Some investigators have reported the effects of L-NAME on cocaine self-administration (Pulvirenti et al., 1996; Orsini et al., 2002). These findings show that NO does not have a direct effect on drug-induced reward. Further experiments may be needed to evaluate the exact role of NO in reinforcing effects of drugs. It has been shown that inhibition of NOS may reduce the conditioned place preference induced by morphine (Biala and Langwinski, 1996; Kivastik et al., 1996; Karami et al., 2002; Gholami et al., 2002; Zarrindast et al., 2002). These results show that NO can modulate the positive reinforcing properties of morphine on the mesolimbic dopamine system. NO acts as a link for glutamate action in the brain (Kiss and Vizi, 2001) and glutamate are an important mediator for the action of morphine in the brain (for example, see Tzschentke and Schmidt, 1997, 1998) and it is therefore possible that complex interactions occur between morphine and NO. Therefore, it seems that NO is necessary for the action of morphine in mesolimbic dopamine areas for the expression of its positive reinforcing properties including self-administration. In spite of the relations of morphine reward and dopamine, some investigators have suggested that other mechanisms may be involved in morphine reward (Koob and Bloom, 1988; Pettit et al., 1984; Van Ree and Ramsey, 1987). To clarify the exact mechanism(s) involved, further experiments may be required.

Acknowledgements

This study was supported by a grant from Baghyatallah (a.s.) University of Medical Sciences, Research Department and Behavioral Science Research Center (BSRC).

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