

Evaluation of Nitric Oxide Involvement in Effect of Lead on Dependency to Morphine in Mice

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ABSTRACT

In the present study, interactions between lead exposure with nitric oxide precursor (L-arginine) or nitric oxide synthase (NOS) inhibitor (L-NAME) on naloxone-induced jumping and diarrhea in morphine-dependent mice were examined. Chronic lead acetate (0.05%) exposure altered naloxone-induced jumping and diarrhea in mice. Jumping was decreased after 7 days and was unchanged 14 and 28 days after lead exposure. Diarrhea was only increased 28 days after lead exposure, which shows a difference between two signs of withdrawal syndrome. Since jumping is the most important sign, the animals were exposed to lead for 7 days in the rest of experiments. In a set of experiments, the nitric oxide agents (L-arginine) or L-NG-nitro arginine methyl ester (L-NAME) were used before naloxone injection to test their effects on the expression of jumping. The low dose of L-arginine, a precursor of nitric oxide (20 mg/kg) decreased jumping, but increased diarrhea. Higher dose of L-arginine (80 mg/kg) increased jumping, while decreased diarrhea. L-NAME decreased both jumping and diarrhea. On the other hand, L-arginine in combination with lead reversed lead-induced attenuation of naloxone-induced jumping, while decreased diarrhea. L-NAME in combination with lead decreased diarrhea, while did not alter jumping. In the second set of experiments, nitric oxide drugs were injected during development of morphine dependency. Data showed that jumping was increased or decreased by low or higher dose of L-arginine respectively. Diarrhea was also increased by the drug. L-NAME decreased both jumping and diarrhea in the development of morphine dependency. Both L-arginine and L-NAME in combination with lead decreased lead-induced jumping and diarrhea. It is concluded that nitric oxide may modulate morphine withdrawal signs and lead-induced attenuation of jumping.

Key Words:

Nitric Oxide,
Lead,
Dependency,
Mice

1. Introduction

Lead has been known to be the most poisonous metal and is widely distributed in the environment. Exposure to lead can occur via food, water and inhaled through the lungs (1). Toxic effects of lead are noted in the kidney, nervous, hematopoietic and gastrointestinal systems (2). Many biological alterations produced by

lead appear related to the ability of the metal either to inhibit or mimic the action of calcium. Lead ions (Pb^{2+}) antagonize the action of Calcium ions at many sites and also mimic many of the biological effects of Calcium ions, for example it interacts with many Calcium binding sites, such as with calmodulin or the Ca^{2+} -dependent K^+ permeability in human blood cell (3). Various neurotransmitter systems including dopaminergic (4), noradrenergic (5-6), cholinergic (7-8) and GABAergic (9) may be affected by

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this metal. Lead ions exert multiple effects on opiate receptor binding sites and are likely to regulate the function of opiate receptors *in vivo* (10-11). Treatment of nursing rats with lead acetate resulted in a small but significant increase in morphine-seeking behavior in the adult offspring of these animals (12). Lead has been shown to alter nitric oxide production in rat brain leading to neuronal dysfunction (13). Nitric oxide has also been proposed to play a role in expression of morphine tolerance and dependence (14). In the present study, effects of lead acetate and/or nitric oxide agents (NO-agents) on the expression and development of dependency to morphine in mice were investigated.

2. Methods

2.1. Animals

Male NMRI mice (20-30 g) were housed in plastic cages in an animal room maintained at 22-25 °C on a 12/12-h light/dark cycle. Food and water was available at all times except during the experiments. Each animal was used once and was killed immediately after the experiment. The protocol was approved by Ethical Committee of Faculty of Pharmacy, Tehran University of Medical Science (No 769; Aug 9, 1999).

2.2. Induction of Dependency

The mice were rendered dependent on morphine, based on the method we used previously (15). Morphine sulfate was injected subcutaneously (s.c.) three times daily at 8 and 12 a.m. and 16 p.m. on the following dosage schedule. The first three doses were 50, 50 and 75 mg/kg respectively. The higher dose of the third daily injection was aimed to minimize any overnight withdrawal. A dose of 50 mg/kg of morphine sulfate was also injected on 4th day (2 h before naloxone injection). Hyperactivity and the Straub tail effect were seen after morphine injections.

2.3. Naloxone-Induced Jumping

Groups of 7 mice each were tested for the occurrence of jumping after their tenth injection of morphine on day 4. Two hours after the last dose of morphine (50 mg/kg), abstinence was precipitated by an intraperitoneal (i.p.) injection of naloxone (5 mg/kg); then the animals were placed individually in a Perspex observation cylinder (15 cm diameter and 50 cm height). The number of jumps was recorded immediately after injection of naloxone over a 30-min period.

2.4. Drugs

The following drugs were used: morphine sulphate (Temad Co., Iran), L-arginine, L-NAME (NG-nitro-

L-arginine methyl ester), sodium acetate, lead acetate (Sigma Chemical Co, USA). Volume of 10 ml/kg, except morphine, which was administered subcutaneously (s.c.). The control groups received saline.

2.5. Drug Treatment

The animals received 10 injections of morphine as described earlier, in order to develop dependence to morphine. The number of jumps induced by naloxone was compared to those that received 10 injections of saline instead of morphine. All animals in experiments 1-7 were morphine-dependent mice. The animals except those in experiments 2 and 6 were also exposed to lead (0.05%) or sodium acetate (0.025%) in drinking water. The drugs were injected either before naloxone administration (effect of drugs on expression) or during development to morphine (effect of drugs on development). The results are mentioned in the results section.

2.6. Experiment 1

The morphine-dependent animals were exposed to lead acetate (0.05%) or sodium acetate (0.025%) in drinking water for 7, 14 and 28 days. The results are shown in Fig. 1.

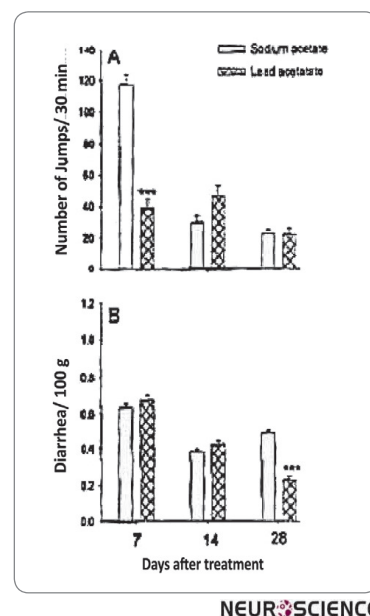


Figure 1. Effects of lead on the development of dependency to morphine. The animals received lead acetate (0.05%) or sodium acetate (0.025%) for 7, 14 or 28 days as well as morphine (s.c.) 3 times daily for last 3 days of lead or sodium acetate exposure in order to induce dependency to morphine. All the dependent animals received naloxone (5 mg/kg, i.p.) to induce jumping and diarrhea. Jumping (Fig. 1A) and diarrhea (Fig. 1B) were recorded for 30 min immediately after naloxone injection. Each group comprised 7 mice. Data are means \pm S.E.M. *** $p < 0.001$ different from the sodium acetate control group

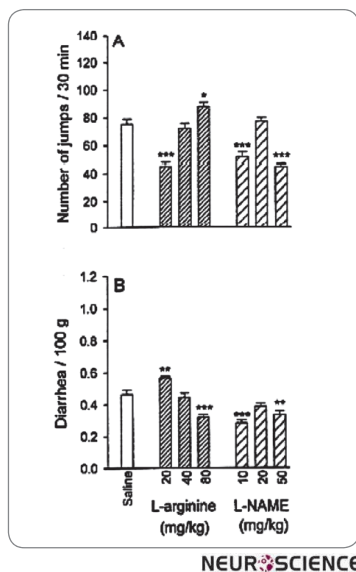


Figure 2. Effects of L-arginine (a precursor of nitric oxide) and L-NAME (nitric oxide synthase inhibitors) on the expression of morphine dependency in mice. All animals received morphine (s.c.) 3 times daily for 3 days, in order to induce dependency to morphine. All the dependent animals received naloxone (5 mg/kg, i.p.) to induce jumping and diarrhea. L-arginine (20, 40, 80 mg/kg, i.p.) and L-NAME (10, 20, 50 mg/kg, i.p.) were injected 30 min before naloxone on the day 4, then jumping. Data are means \pm S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ different from the saline control group

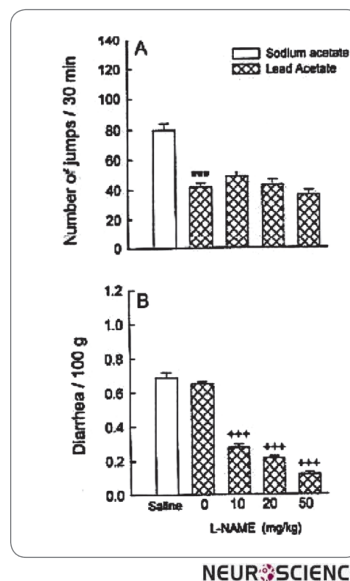


Figure 4. Effects of L-NAME on the expression of morphine dependency in mice. All animals received morphine (s.c.) 3 times daily for 3 days, in order to induce dependency to morphine. All the dependent animals received naloxone (5mg/kg, i.p.) to induce jumping and diarrhea. L-NAME (10, 20, 50 mg/kg, i.p.) was injected 30 min before naloxone injection on the day 4. Jumping (Fig. 4A) and diarrhea (Fig. 4B) were recorded for 30 min immediately after naloxone injection. Each group comprised 7 mice. Data are means \pm S.E.M. *** $p < 0.001$ different from respective sodium acetate control group. +++ $p < 0.001$ different from respective L-NAME control group.

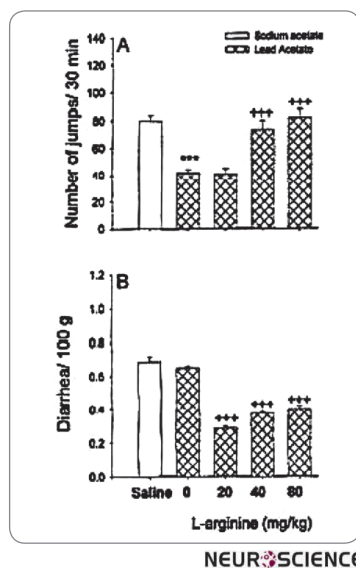


Figure 3. Effects of L-arginine on the expression of morphine dependency in mice. All animals received morphine (s.c.) 3 times daily for 3 days, in order to induce dependency to morphine. All the dependent animals received naloxone (5 mg/kg, i.p.) to induce jumping and diarrhea. L-arginine (20, 40, 80 mg/kg, i.p.) was injected 30 min before naloxone injection on the day 4. Jumping (Fig. 3A) and diarrhea (Fig. 3B) were recorded for 30 min. Each group comprised 7 mice. Data are means \pm S.E.M. *** $p < 0.0001$ different from respective sodium acetate control group. +++ $p < 0.0001$ different from respective L-arginine control group

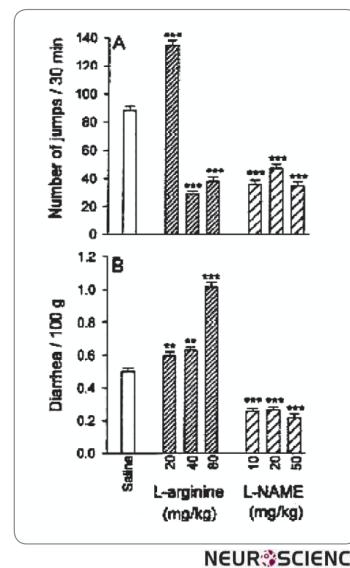


Figure 5. Effects of L-arginine (a precursor of nitric oxide) and L-NAME on the development of morphine dependency in mice. All animals received morphine (s.c.) 3 times daily for 3 days, in order to induce dependency to morphine. All the dependent animals received naloxone (5 mg/kg, i.p.) to induce jumping and diarrhea. L-arginine (20, 40, 80 mg/kg, i.p.) and L-NAME (10, 20, 50 mg/kg, i.p.) were injected 30 min before morphine 3 days (during development of dependency). Jumping (Fig. 5A) and diarrhea (Fig. 5B) were recorded on the day 4 immediately after naloxone injection for period of 30 min. Each group comprised 7 mice. Data are means \pm S.E.M. ** $P < 0.01$, *** $P < 0.001$ different from the saline control group

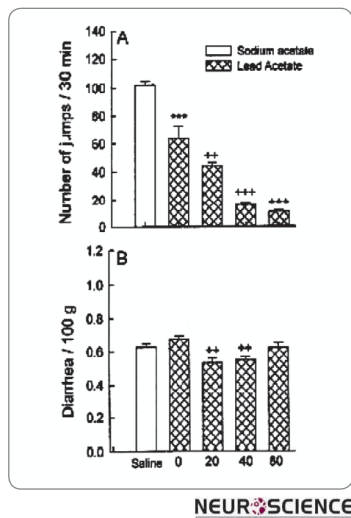


Figure 6. Effects of L-arginine on the development of morphine dependency in mice. All animals received morphine (s.c.) 3 times daily for 3 days, in order to induce dependency to morphine. All the dependent animals received naloxone (5mg/kg, i.p.) to induce jumping and diarrhea. L-arginine (20, 40, 80 mg/kg, i.p.) was injected 30 min before morphine 3 days (during development of dependency). Jumping (Fig. 6A) and diarrhea (Fig. 6B) were recorded on the day 4 immediately after naloxone injection for period of 30 min. Each group comprised 7 mice. Data are means \pm S.E.M. *** $p < 0.001$ different from respective sodium acetate control group. ** $p < 0.01$, +++ $p < 0.001$ different from respective L-arginine control group

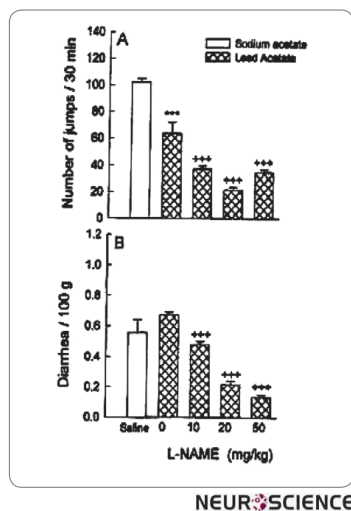


Figure 7. Effects of L-NAME on the development of morphine dependency in mice. All animals received morphine (s.c.) times daily for 3 days, in order to induce dependency to morphine. All the dependent animals received naloxone (5mg/kg, i.p.) to induce jumping and diarrhea. L-NAME (10, 20, 50 mg/kg, i.p.) was injected 30 min before morphine 3 days (during development of dependency). Jumping (Fig. 7A) and diarrhea (Fig. 7B) were recorded on the day 4 immediately after naloxone injection for period of 30 min. Each group comprised 7 mice. Data are means \pm S.E.M. *** $p < 0.001$ different from respective sodium acetate control group. +++ $p < 0.001$ different from respective L-NAME control group.

2.7. Experiment 2

The morphine-dependent animals received saline, NO precursor (L-arginine) or NO synthase inhibitor (L-NAME), before naloxone injection (before expression of jumping). The results are shown in Fig. 2.

2.8. Experiment 3

The morphine-dependent animals received saline (10 ml/kg) or different doses of L-arginine (20-80 mg/kg) 30 min before naloxone injection to test the response of the drug on expression of withdrawal signs. The animals were exposed to sodium acetate or lead acetate in drinking water. The results have been shown in Fig. 3.

2.9. Experiment 4

The morphine-dependent animals received saline (10 ml/kg) or different doses of L-NAME (10-50 mg/kg) 30 min before naloxone injection to test the response of the drug on expression of withdrawal signs. The animals were exposed to sodium acetate or lead acetate in drinking water. The data have been shown in Fig. 4.

2.10. Experiment 5

The morphine-dependent animals received saline, NO precursor (L-arginine) or NO synthase inhibitor (L-NAME), 30 min before morphine, 3 times a day for 3 days during development of dependency (Fig. 5).

2.11. Experiment 6

The morphine-dependent animals received saline (10 ml/kg) or different doses of L-arginine (20-80 mg/kg) 30 min before morphine, 3 times a day for 3 days, during development of dependency, in order to test effect of L-arginine on development of dependency to morphine. The animals were exposed to sodium acetate or lead acetate in drinking water (Fig. 6).

2.12. Experiment 7

The morphine-dependent animals received saline (10 ml/kg) or different doses of L-NAME (10-50 mg/kg) 30 min before morphine, 3 times a day for 3 days.

2.13. Statistical analysis

Analyses of variance (ANOVA) followed by Newman-test were used for analysis of the data. Differences between means were considered statistically significant if $p < 0.05$.

3. Results

3.1. Naloxone-Induced Withdrawal Jumping in Morphine-Dependent Mice

The mice were divided randomly into two groups. One group received morphine (as described in Methods) to induce dependency. The next group received saline (10 ml/kg, i.p.) instead of morphine (s.c.). Naloxone (5 mg/kg, i.p.) increased the number of non-dependent mice. The results showed that naloxone could induce jumping in morphine-dependent mice. We considered jumping behavior as the signs of abstinence for further experiments in our study. Hyperactivity and Straub tail reaction were seen after morphine injections.

3.2. Effects of Lead Exposure on Expression of Naloxone- Induced Jumping Behavior or Diarrhea in Morphine Dependent Mice

Fig. 1 indicates effects of lead on development of dependency to morphine. The animals received i.p. injection of lead acetate (0.05%) or sodium acetate (0.025%) for 7, 14 or 28 days, as well as morphine (s.c.) 3 times daily for last 3 days of lead or sodium acetate exposure, in order to induce dependency to morphine as described earlier in method section. Two-way ANOVA indicates that lead exposure altered naloxone-induced jumping [$F(2,36)=47.0$, $p<0.0001$] and diarrhea [$F(2,36)=8.3$, $p<0.001$]. Analysis shows that jumping was decreased 7 days, was not altered 14 days and 28 days after exposure to lead acetate. Diarrhea was increased 28 days after lead exposure. The results may indicate that chronic lead exposure may disrupt opiate receptor mechanism.

3.3. Effects of No- Agents on Expression of Naloxone- Induced Jumping Behavior or Diarrhea on Morphine-Dependent Mice

Fig. 2 indicates the effects of L-arginine, precursor of NO, L-NAME, NO synthase inhibitor on the expression of morphine dependency in mice. All animals received morphine (s.c.) 3 times daily for 3 days, in order to induce dependency to morphine as described earlier. L-arginine and L-NAME were injected i.p. 30 min. before naloxone on the day 4, then the number of jumps and diarrhea were recorded immediately after naloxone injection for period of 30 min. One-way ANOVA shows a significant difference between jumps [$F(9,60)=20.6$, $p<0.0001$] and diarrhea [$F(9,60)=15.7$, $p<0.0001$] induced by naloxone in the presence or absence of the drugs. Further, analysis shows that jumping was decreased by low dose (20 mg/kg, i.p.) and increased by higher dose (80 mg/kg, i.p.) of L-arginine. L-NAME decreased jumping in expression of jumping. Diarrhea

was increased by lower dose but decreased by higher dose of L-arginine, while decreased by L-NAME.

3.4. Effects of No-Agents on Expression of Naloxone-Induced Jumping Behavior or Diarrhea in The Presence or Absence of Lead Acetate in Morphine-Dependent Mice

All the animals in the following groups (Fig.3 and 4) received i.p. injection of either lead acetate (0.05%) or sodium acetate (0.025%) for 7 days, as well as morphine (s.c.) 3 times daily for last 3 days of lead or sodium acetate exposure, in order to induce dependency to morphine as described earlier in method section. The NO-agents were administered 30 min before naloxone injection on the day 4 of dependency. Jumps and diarrhea were recorded for 30 min.

Fig. 3 shows the effects of L-arginine on the expression of morphine dependency in mice. Two-way ANOVA shows a significant difference between the effects of L-arginine on naloxone-induced jumping [$F(3,48)=30.0$, $p<0.0001$], and diarrhea [$F(3,48)=22.7$, $p<0.0001$] in the presence or absence of lead. Data show that lead decreased jumping. L-arginine reversed lead-induced attenuation of jumping and increased diarrhea.

Fig. 4 shows the effects of L-NAME on the expression of morphine dependency in mice. Two-way ANOVA shows a significant difference between the effects of L-NAME on naloxone-induced jumping [$F(3,48)=11.5$, $p<0.0001$], and diarrhea [$F(3,48)=17.5$, $p<0.0001$] in the presence or absence of lead. Data shows that lead decreased jumping, and L-NAME decreased diarrhea but not jumping.

3.5. Effects of No-Agents on Development of Naloxone- Induced Jumping Behavior or Diarrhea in Morphine-Dependent Mice

Fig.5 indicates the effects of L-arginine and L-NAME on the development of morphine dependency in mice. All animals received morphine (s.c.) 3 times daily for 3 days, in order to induce dependency to morphine. L-arginine and L-NAME were injected 30 min before morphine 3 days (during development of dependency), the number of jumps and diarrhea were recorded on the day 4, immediately after naloxone injection for period of 30 min. One-way ANOVA shows a significant difference between jumps [$F(9,60)=139.7$, $p<0.0001$] and diarrhea [$F(9,60)=138.9$, $p<0.0001$] induced by naloxone in the presence or absence of the drugs. Analysis shows that jumping was increased by low dose (20 mg/kg, i.p.) or decreased by higher doses (40 and 80 mg/kg, i.p.) of L-arginine while, diarrhea was increased by the drug. L-NAME decreased both jumping and diarrhea.

3.6. Effects of No-Agents on Development of Naloxone-Induced Jumping Behavior or Diarrhea in The Presence or Absence of Lead Acetate in Morphine-Dependent Mice

All animals rendered dependent as described. L-arginine and L-NAME were injected 30 min before morphine 3 days (during development of dependency) the number of jumps and diarrhea were recorded on the day 4, immediately after naloxone injection for period of 30 min. Fig. 6 shows the effects of L-arginine on the development of morphine dependency in mice. Two-way ANOVA shows a significant difference between the effects of L-arginine on naloxone-induced jumping [$F(3,48)=34.7, p<0.0001$], and diarrhea ($F(3,48)=5.5, p<0.01$) in the presence or absence of lead. Data show that lead decreased jumping and L-arginine potentiated the response of lead. L-arginine in combination with lead decreased diarrhea.

Fig. 7 shows the effects of L-NAME on the development of morphine dependency in mice. Two-way ANOVA shows a significant difference between the effects of L-NAME on naloxone-induced jumping [$F(3, 48) =5.3, p<0.01$], and diarrhea [$F(3,48)=47.3, p<0.0001$] in the presence or absence of lead. Data show that lead decreased jumping, which was potentiated in the presence of L-NAME. Diarrhea was decreased by low dose of L-NAME.

4. Discussion

The several neurotransmitters including serotonin, dopamine, GABA, adenosine, cholecystokinin and aspartate seem to be involved in morphine tolerance and dependence (16, 17). Mesolimbic dopaminergic neurons are thought to serve as a final common neural pathway for mediating reinforcement processes. Long lasting neuroadaptive changes in mesolimbic dopamine-mediated transmission that develop during chronic drug used might contribute to compulsive drug-seeking behavior and relapse (18). It has also been shown that lead is capable of affecting dopamine receptor subtypes (4). Furthermore, lead exposure also has been found to produce functional dopaminergic supersensitivity, which involves both the dopamine D1 and D2 receptor subtypes (19). The present data show that chronic lead exposure for 7 days but not for 14 or 28 days decreased naloxone-induced jumping. It may be concluded that after first week of the lead exposure tolerance can be elicited. This is also in agreement with our previous results that lead exposure reduced jumping (20). Since dopaminergic mechanism has been implicated in naloxone-induced jumping in morphine-dependent mice (15), and lead exposure may disrupt dopaminergic (4, 19), one site of interactions may be dopamine receptors. Furthermore, it has been shown that nitrite and nitrate increased after 7 days but not after 14 days of lead exposure (13). Since increase in NO level, during develop-

ment of opioid dependency may attenuate jumping. (14), this may explain why only lead reduced jumping after 7 days of lead exposure. One important area that is thought to represent a site of origin of the opioid withdrawal syndrome is the locus coeruleus and nucleus accumbens (21-23). However, the role of locus coeruleus in the expression of opioid withdrawal is controversial (24). It has been suggested that chronic opiate administration induce different adaptations in the locus coeruleus, which some of these changes appear to be relevant in the behavioral consequences of opiate dependence and withdrawal (25). Opiate withdrawal might be mediated by nitric oxide acting as an intermediate messenger in the locus coeruleus (26). Our present study showed that pretreatment of animals with low dose (20 mg/kg) and higher dose (80 mg/kg) of L-arginine before naloxone administration (expression of jumping) decreased or increased expression of jumping respectively.

The present data have shown that L-arginine in combination with lead reversed lead-induced attenuation of naloxone-induced jumping and decreased diarrhea. Lead has also been shown to inhibit calcium-stimulated nitric oxide synthase activity in the rat cerebellum (27). Therefore opposite response of L-arginine in the presence or absence of lead may indicate that change in nitric oxide levels elicit a modulation of withdrawal jumping. NO induces release of neurotransmitters by calcium dependent and independent mechanisms, including acetylcholine, dopamine, norepinephrine, GABA, excitatory amino acids, etc (28). Furthermore, NO exerts a dual effect on the neurotransmitter release. For example, low dose of the agent decrease release of dopamine while, high concentration of NO elevates the neurotransmitter (29). So, these controversial responses can be due to the dual effect of the agent. L-NAME decreased diarrhea. The similar responses of some doses of L-arginine and L-NAME in expression of naloxone-induced withdrawal signs, in this study may also further support the hypothesis of modulator role of nitric oxide in withdrawal.

Administration of lower dose and higher dose of L-arginine, during induction (development) of morphine dependency, in contrast to the effect of the drug when was used before expression, increased or decreased jumping respectively and increased diarrhea. Decrease in nitric oxide levels with L-NAME decreased both jumping and diarrhea. However, there is a report (14) indicating that increase or decrease in nitric oxide levels during development of opioid dependency elicited attenuation or acceleration of jumping respectively, our data may support the hypothesis that nitric oxide induces modulation of jumping withdrawal.

The present study showed that L-arginine potentiated the lead-induced attenuation of naloxone-induced jumping, and diarrhea decreased by L-arginine in combination

with lead. Lead-induced attenuation of jumping also was potentiated in the presence of L-NAME, while diarrhea was decreased by the low dose of the drug. These data also show that nitric oxide may involve in development of dependency and lead-induced attenuation of jumping.

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