

EFFECT OF L- ARGININE, THE NITRIC OXIDE PRECURSOR, ON MORPHINE TOLERANCE IN OVARIECTOMIZED RATS

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Summary

In present study probable effects of L- arginine on morphine tolerance in ovariectomized rats was investigated. 8 weeks after ovariectomy, rats were divided into test groups, including Tolerant and Tolerant+ L-Arginine(10, 50 and 200 mg/kg) groups. Morphine tolerance was induced by daily injection of 20 mg/kg morphine during 4 consecutive days. Animals of groups 2, 3 and 4 were simultaneously treated with 10, 50 and 200mg/kg L-Arginine, respectively. Hotplate test was carried out in 5th day as a base record, then the animals received 5 mg/kg morphine and antinociceptive effect was evaluated every 10 min. The base time of Tolerant group was lower than Tolerant + L-Arginine 10, 50 and 200 mg/kg groups. Analyzing by repeated measure ANOVA showed that reaction time after last injection of 5 mg/kg morphine in tolerant group was lower than all 3 Tolerant + L-Arginine groups .This implies that L-Arginine reduces morphine tolerance in ovariectomized rats.

Key words: L- Arginine, Tolerance, Morphine, Ovariectomized rat

Running title: L-arginine and morphine tolerance in ovariectomized rats

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Introduction

Opiates, mainly morphine are amongst most effective antinociceptive drugs which are commonly used to relieve pain (1). Although uses of opiates are restricted due to the development of tolerance and physical or mental dependency to these drugs (1). It has been documented that some neuroendocrine systems including gonadal hormones may change development of tolerance or dependency to these drugs (2).

According to some studies, sex differences may change the response to opiates including morphine, in human cases and in laboratory animals. In general, males present higher level of antinociception than females. In case of animals, male rats show greater tolerance to morphine in comparison with female rats (3,4,5,6,7). Although the mechanism by which sex differences interrupt morphine tolerance is almost unknown, but lots of studies performed to investigate the probable role of estrogen in this regard. Noticeably the interaction between estrogen and NO system may be responsible about morphine tolerance.

It has been suggested that NO as a major neurotransmitter is involved in nociceptive processes either in the periphery or within the spinal cord (8). L-arginine, a nitric oxide precursor, reduces the antinociceptive effect of morphine (9), whereas the constitutive nitric oxide synthase (cNOS) inhibitors potentiate the morphine analgesia in the tail-flick test (10,11). On the other hand, the results of another study imply that acute administration of L-arginine, the precursor of NO, p.o. or i.p. but not i.c.v. reduced morphine antinociception in mice. These effects were reversed by the NOS inhibitors L-NAME and L-NMMA (12). Intrathecal administration of L-NAME has also been shown to enhance morphine antinociception in rats (13), suggesting involvement of the spinal NO system. Inhibition of the spinal NO synthase subsequently potentiates the mu-, delta- and to a lesser extent, kappa opioid receptors-mediated spinal antinociception in both acute and prolonged pain. Moreover it has been demonstrated that cNOS inhibitors reduce tolerance developed to the analgesic effect of morphine (14,15). Altogether, inhibition of NO synthesis and blocking of morphine tolerance result in enhancement of

morphine antinociception which suggest a selective action of NO in the mechanisms of mu receptor-mediated tolerance and dependence (14,16,17).

Estrogen has been shown to influence the NO system, it increases NO production (18,19), endothelial nitric oxide synthase (eNOS) expression (18,20), and eNOS activity (18,19) in cultured endothelial cells. Estrogen also increases eNOS protein expression in cerebral microvessels (21, 22, 23, 24). Finally, estrogen increases NO production (25,26) and eNOS protein expression (27) in ex vivo preparations of the median eminence. Estrogen increases the numbers of NADPH-diaphorase (neuronal NOS [nNOS]) neurons (28,29) and nNOS mRNA (30,31) in selected brain regions of OVX rats. Some other studies report that estrogen does not change nNOS mRNA levels or numbers of nNOS neurons in other brain regions (30,32). The present study carried out to evaluate the effect of L-arginine on tolerance to morphine in OVX rats.

Material and Methods

Animals and drugs

32 Female wistar rats (200±10 gr) were used. All rats were housed in 4–6 per standard cages, at room temperature (24± 1 °C) on a 12 h light/dark cycle. Food and water were available properly. Animal handling and all related procedures were in accordance with approved standards of animal caring.

Drugs used were L-arginine(Sigma, USA), morphine sulfate (TEMAD Ltd., Teheran, Iran), ketamine (Daroo-Pakhsh Pharma, Iran). All drugs were dissolved in saline solution. Injections were all intraperitoneal.

Surgery

Rats were ovariectomized under ketamine anesthesia (150 mg/kg, i.p.) (33). Anesthesia was confirmed by reduced respiratory rate and no response to gentle pinching of foot pad. Ventral incision was made through the skin of the flank of the rat and ovaries and ovarian fats were removed. Ovaries were isolated by ligation of the most proximal portion of the oviduct before removal.

Tolerance induction

Two months after surgery, tolerance was induced through daily morphine injection (20 mg/kg, i.p) during 4 days(34). Rats received a single injection of 5 mg/kg morphine 24 h after last injection and hot plate test was carried out.

Nociceptive test

To assess nociceptive responses, hot plate method was used. In hot plate method, rats were placed on the with temperature setting controlled at 52 ± 0.2 °C. Cut-off time was 60 seconds. Nociceptive response is defined as licking fore paws or moving hind paws. Time duration between placing animals on hot plate and licking fore paws or moving hind paws is considered as reaction time. The hot plate test was performed as a base record 10 min before last injection of morphine (5 mg/kg) and consequently it was repeated every 10 minutes after injection.

Experimental design

Thirty two OVX rats were divided into four groups: 1- Tolerant group (Tol): morphine tolerance was induced as described above. 2- Tolerant + L-Arginine 10 mg/kg (Tol+L-A10): morphine tolerance was induced similarly. The animals of this group were treated with L- Arginine (10 mg/kg) during 4 days of tolerance induction. 3- Tolerant + L-Arginine 50 mg/kg (Tol+L-A50): morphine tolerance was induced similarly. The animals of this group were treated with L- Arginine (50 mg/kg) during 4 days of tolerance induction. 4- Tolerant + L-Arginine 200 mg/kg (Tol+L-A200): morphine tolerance was induced similarly. The animals of this group were treated with L- Arginine (200 mg/kg) during 4 days of tolerance induction.

Procedure

Ovariectomized rats, categorized into 4 groups, 8 weeks after surgery. Morphine tolerance was induced by daily injection of 20 mg/kg morphine during 4 consecutive days. Animals of groups 2, 3 and 4 were simultaneously treated with 10, 50 and 200mg/kg L-Arginine, respectively. Hotplate test was carried out in 5th day as a base

record, then the animals received 5 mg/kg morphine and antinociceptive effect was evaluated every 10 min.

Statistical analysis

All data were presented as mean \pm S.E.M of reaction time. Statistical comparison of base reaction time between groups was done with one-way analysis of variance (ANOVA). Repeated measure ANOVA followed by post hoc tukey test was used for comparison of reaction times after injection of morphine. Differences were considered statistically significant when $p < 0.05$.

Results

The results of the hot-plate test indicate two facts:

- 1- As the fig 1 shows, the basal reaction time of Tolerant group was 27 ± 1.41 sec. and it was increased respectively up to 46.77 ± 3.52 , 51.75 ± 2.81 and 47.9 ± 2.78 sec. in Tolerant + L-Arginine 10, 50 and 200mg/kg groups respectively. The interesting point is that increase in hot plate reaction time, was significantly higher in tolerant+ L-Arginine groups compared with tolerant group ($P < 0.001$). there was no difference between L-Arginine groups ($P > 0.05$).
- 2- Analyzing by repeated measure ANOVA showed that reaction time after last injection of 5 mg/kg morphine in tolerant group was lower than Tolerant + L-Arginine 10 ,50 and 200mg/kg groups ($P < 0.001$)(fig 2). This implies that L-Arginine reduce morphine tolerance in ovariectomized rats.

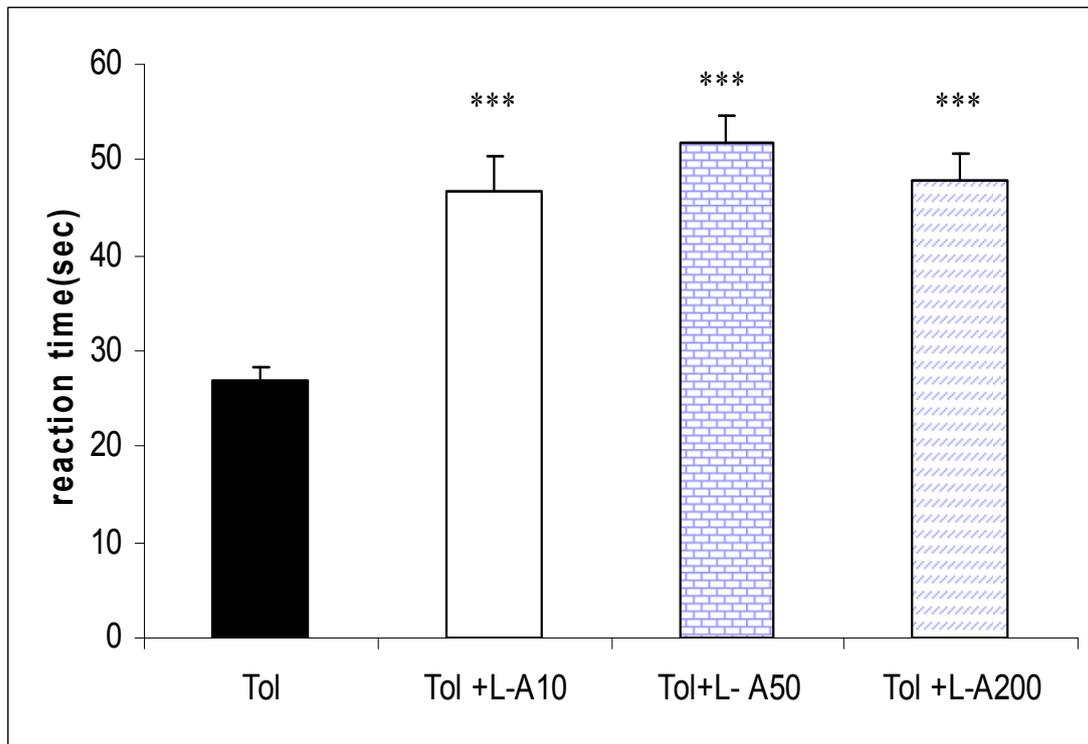


Fig 1: Comparison of base time between Tolerant and Tolerant + L-arginine 10, 50 and 200mg/kg groups(8 animals each).

Tol: Tolerant group, Tol + L-A10: Tolerant + L-arginine 10mg/kg group, L-A 50: Tolerant + L-arginine 50mg/kg group, L-A 200: Tolerant + L-arginine 200mg/kg group

Rats were tolerated by injection of 20 mg/kg of morphine during 4 consecutive days

Base time was recorded in 5th day and compared among 4 groups using one way ANOVA.***P<0.001 compared to Tolerant group

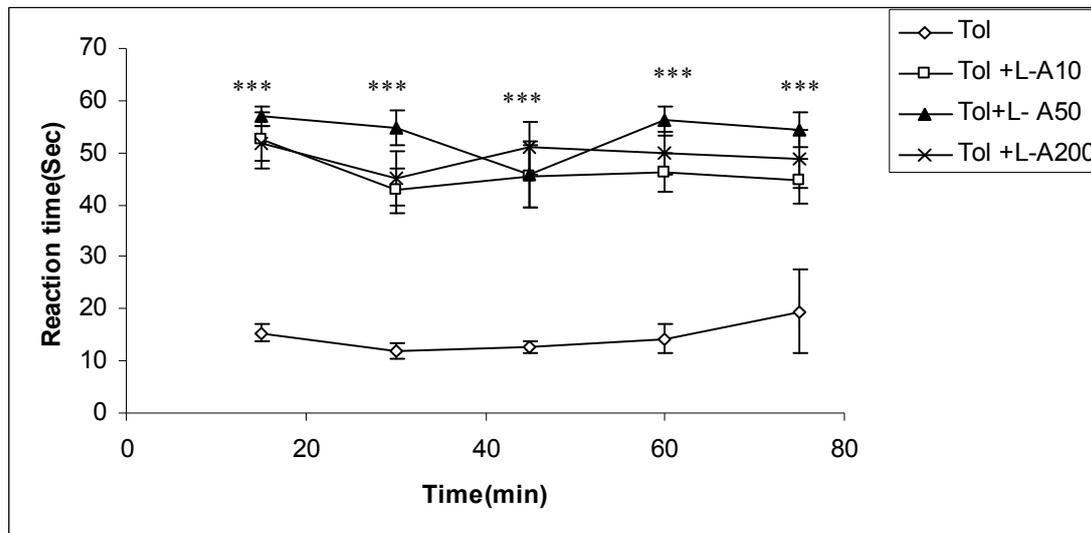


Fig 2: Comparison of reaction times after 5mg/kg morphine injection between Tolerant and Tolerant + L-arginine 10, 50 and 200mg/kg groups (each group includes 8 animals).

Tol: Tolerant group, Tol + L-A 10: Tolerant + L-arginine 10mg/kg group, L-A 50: Tolerant + L-arginine 50mg/kg group, L-A 200: Tolerant + L-arginine 200mg/kg group

Tolerance was induced by injection of 20 mg/kg of morphine during 4 consecutive days. 5 mg/kg of morphine injected in 5th day and reaction times were recorded every 10 min. Comparison between 4 groups was performed through using repeated measure ANOVA. Reaction time after last injection of 5 mg/kg morphine in tolerant group was lower than Tolerant + L-arginine 10, 50 and 200mg/kg groups ($P < 0.001$)

Discussion

In the present study the effect of L- arginine on tolerance procedure in ovariectomized rats, using hot plate test was investigated. Hot plate test used in the present study, is a well known standard method for pain threshold evaluation after morphine or other analgesic drugs administration (35). The results showed that in the absence of physiological level of estrogen, L-arginine a precursor of NO attenuates morphine tolerance.

Production of nitric oxide (NO), a versatile molecule with various roles in signaling processes, memory formation, and pain perception is highly dependent to synthesis, catabolism and transport of arginine. NO is synthesized by 3 isoforms of nitric oxide synthase (NOS) (36). Different studies confirmed the role of NO as a neuronal messenger in nociceptive processes, as it was presented that in rat studies, after nociceptive visceral stimulation, NOS activity was significantly increased in the ventrolateral areas of the periaqueductal gray matter (PAG). Relevantly, the electrical stimulation of dorsolateral PAG provoked acceptable analgesic effects, which was attenuated by serotonin. L-NAME was shown to block the inhibitory effects of serotonin in PAG (37). Although the mechanism involved in the development and expression of opioids tolerance and dependence is still unclear but there are raising evidences of NO system involvement. It was indicated that elevated level of NO during tolerance induction (using L-arginine) delays the development of opioid tolerance and dependence, while reducing NO levels (with L-NAME and L-NMMA) accelerates the development of tolerance with no effect on that of opioid dependence (37). The result of our experiment is in accordance with this statement; however, current study was performed on ovariectomized rats. Majority of studies consider NO to have an analgesic action. Therefore, increased production of NO as a result of chronic administration of L-arginine would result in antagonism of the antinociceptive response to morphine, as was observed in current study (38, 39). It has been suggested that L-arginine plays an antinociceptive role in the brain, via the kyotorphin-Met-enkephalin pathway(40). It seems that L-arginine is a constituent amino acid of kyotorphin (L-tyrosyl-L-arginine), an endogenous met- enkephaline releaser in the brain(40). This pathway may also be involve in the result of present study. Chung et al reported that antinociceptive action of L-ARG are mediated by dynorphin(41). we suggest the production of dynorphin as possible mechanism for the result of present study. The result of present study is in sharp contrast to the findings reported by Babey et al. (1994) that L-arginine 'induces' tolerance in opioid naive mice through NOS, and accelerates tolerance when co-administered with morphine, while NOS inhibitors prevent morphine tolerance(39). Other findings support the view that tolerance disappears or diminishes after the central inhibition of NO synthase and increases after central NO synthase activation (42). Investigation which has been done on rat hippocampal slices,

demonstrated that drugs which suppressed the synthesis or release of NO would be expected to block the development of morphine tolerance (43). In conclusion the result of present study showed that L-arginine attenuates development of tolerance to morphine in ovariectomized rats.

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