

EFFECTS OF KETAMINE AND MAGNESIUM ON MORPHINE INDUCED TOLERANCE AND DEPENDENCE IN MICE

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ABSTRACT

The goal of this study was to evaluate the effects of ketamine and magnesium on prevention of development of morphine tolerance and dependence in mice. In this study different groups of mice received morphine (50 mg/kg, sc) + (saline 10ml/kg), morphine (50 mg/kg, sc) + ketamine (25,50 or 75 mg/kg, ip), morphine (50 mg/kg, sc) + magnesium (10,20 or 40 mg/kg, ip), morphine (50 mg/kg, sc) + ketamine (25 mg/kg, ip) + magnesium (10 mg/kg, ip)] once a day for four days. Tolerance was assessed by administration of morphine (9 mg/kg, ip) and using hot plate test on fifth day. Withdrawal symptoms were assessed by administration of naloxone (4 mg/kg, ip) two hours after co-administration of morphine with either ketamine or magnesium.

It was found that pretreatment with ketamine or magnesium decreased the degree of tolerance and dependence. Additionally, co-administration of ketamine and magnesium before morphine administration decreased the tolerance and dependence significantly. From these results it may be concluded that administration of ketamine or magnesium alone or together could prevent the development of tolerance and dependence to the analgesic effects of morphine. These effects may be related to the N-Methyl-D-Aspartate (NMDA) receptor antagonist behavior of ketamine and the ability of magnesium to block the Ca channel of NMDA receptors.

Keywords: Morphine, Tolerance, Withdrawal, Magnesium, Ketamine.

INTRODUCTIN

Opiate analgesics such as morphine are widely used in the management of pain. Repeated use of opiates may lead to development of tolerance and dependence (1-3). Tolerance is indicated by a decrease in the efficacy of the drug after chronic use which may leads to the requirement for higher doses to get the desired analgesic effect. Dependence is continued need for the drug to maintain a state of physical equilibrium following repeated use of the drug, and is evidenced by withdrawal manifestation when drug administration is terminated. These phenomena limit the therapeutic efficacy of opioids (4-5). Chronic opioid treatment leads to protein kinase C (PKC) activation and translocation which phosphorylates the NMDA receptor-gated Ca channel, and results in potentiation of NMDA receptor activity (1,3,5-7). Opening of these channels leads to an influx and increases intracellular Ca concentration which produces several effects. NMDA receptor antagonists such as ketamine, have been reported to be able to block the development of morphine tolerance and dependence (6-15). On the other hand it is known that, NMDA receptors are normally blocked by magnesium) at the rest membrane potentials (5,16,17). However chronic use of opiate leads to

opening of channels and increase in intracellular Ca concentration (1,3,5). Recent studies have shown the efficacy of magnesium as a NMDA Ca channel blocker which affects morphine tolerance and dependence (16-18). Other studies have shown that Magnesium could increase the antinociceptive effect of low doses of morphine in chronic and neuropathic pains (17,19). On the basis the NMDA, antagonist behaviors of ketamine and magnesium properties in blocking the Ca channel of NMDA a combination of these agents were administrated to evaluate the possible effects of this combination on morphine tolerance and dependence.

MATERIALS AND METHODS

Animals:

Male albino mice (20-30g) were used. Animals were obtained from the razy Inittue of Iran. Pain sensitivity was measured by hot-plate test.

Drugs:

Morphine sulfate (Darupakhsh - Iran), ketamine hydrochloride (Rotexmedica - Germany), Magnesium sulfate (Darupakhsh - Iran), Naloxone Hydrochloride (Tolid daru- Iran) were used in this study.

Methods

Hot - plate test:

Each animal was placed on a surface (23 × 23 Cm) maintained at 55 ± 2 °C surrounded by a Plexiglas wall of 20 cm high. Licking of hands was used as the end point for determination of the response latencies. Failure to respond after 45 seconds was used as an indicator of termination of the test (cut off).

Induction of tolerance:

In order to induce tolerance, groups of 9 mice were chosen randomly. Mice were treated subcutaneously (sc) with morphine (50 mg/kg) in combination with either ketamine (25,50 or 75 mg/kg, ip) or magnesium (10,20 or 40 mg/kg, ip) or both ketamine and magnesium [(ketamine 25 mg/kg, ip) + magnesium (10 mg/kg, ip)] once a day for four days. To evaluate the degree of tolerance, the antinociceptive effects of a test dose of morphine (9 mg/kg) was measured 24 hours after the last dose of morphine in combination with either ketamine or magnesium alone or together.

Induction of dependence:

Groups of 9 mice were chosen randomly. Mice were treated subcutaneously (sc) with morphine (50mg/kg) in combination with either ketamine or magnesium or both ketamine and magnesium once a day for four days. To evaluate the effect of different doses of ketamine and magnesium in dependence (jumping and standing on feet) a dose of naloxone (4 mg/kg, ip) was injected 2 hours after the last dose of morphine in the fourth day.

Evaluation of the withdrawal syndrome:

After naloxone injection, withdrawal symptoms (number of jumping and number of standing on feet) during 30 min were evaluated.

Statistical Analysis

The results are expressed as the Mean \pm SE. Differences between the individual mean values of different groups were analyzed by one-way analysis of variance (ANOVA) and tukey test as a post hoc analysis and differences with a $p < 0.05$ were considered significant.

RESULTS

Development of tolerance to the morphine antinociception:

Animals received morphine (50 mg/kg, sc) for 2, 3 or 4 days. In each group antinociceptive response of a test dose of morphine (9 mg/kg, ip) was assayed 24 hours after the last dose of morphine (50 mg/kg, sc). Effects of morphine (9 mg/kg, ip)

on tolerant and non tolerant mice were evaluated. Animals that became tolerant to effects of morphine in the fourth day exhibited only a small antinociceptive effect ($p < 0.001$) (Fig.1).

Naloxone-induced withdrawal:

Animals were rendered dependent to morphine by administration of morphine (50 mg/kg, sc) once a day for four days. The dose of 4mg/kg of naloxone was chosen for induction of withdrawal symptoms. Naloxone induced withdrawal signs such as jumping and standing on feet (Fig. 5 and 6).

Effect of pretreatment with ketamine on tolerance and dependence of chronic administration of morphine:

As shown in figure.2, ketamine injection (25, 50 or 75 mg/kg, ip) 30 min before daily morphine administration decreased tolerance to the analgesic effects of morphine significantly ($P < 0.001$). Figures 5 and 6 show that pretreatment with ketamine (25,50 or 75 mg/kg, ip) significantly decreased the withdrawal symptoms in a dose dependent manner ($P < 0.001$).

Effect of pretreatment with magnesium on tolerance and dependence to chronic morphine therapy:

As shown in figure.3, injection of magnesium (10, 20 or 40 mg/kg, ip) 30 min before daily morphine administration decreased tolerance to the analgesic effect of morphine significantly ($P < 0.001$). Figures.5 and 6 show that pretreatment with magnesium (10, 20 or 40 mg/kg, ip) significantly decreased the withdrawal symptoms in a dose dependent manner ($P < 0.001$).

Effect of pretreatment with ketamine and magnesium on tolerance and dependence to chronic morphine therapy:

As it is shown in figure 4, co-administration of ketamine (25 mg/kg, ip) and magnesium (10 mg/kg, ip) 30 min before daily morphine administration decreased tolerance phenomenon significantly ($P < 0.001$), and as it is shown in figures 5 and 6 this combination decreased withdrawal symptoms significantly ($P < 0.001$).

DISCUSSION

The main goal of this study was to evaluate the effects of ketamine as a non competitive NMDA receptor antagonist and magnesium as a NMDA Ca channel blocker on development of tolerance and dependence. Tolerance and dependence may be viewed as a result of neuronal adaptation which is induced by repeated drug exposure, and NMDA receptors have been consistently implicated in

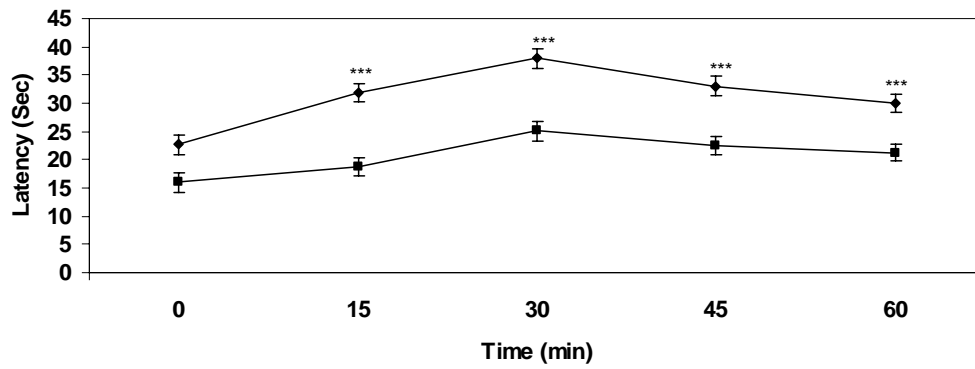


Figure 1. Effects of morphine on tolerant and non-tolerant mice. Animal received either (♦)saline (10 ml/kg, sc) or (■) [morphine (50mg/kg, sc) + saline (10 ml/kg, SC)] for 4 days. Antinociception of a test dose of morphine (9mg/kg, sc) was tested 24 hour after the last dose of morphine (50mg/kg, cs) in tolerant and nontolerant mice. Each group had at least nine mice. Results are expressed as Mean ± SE. *** p<0.001, significantly different from the control group.

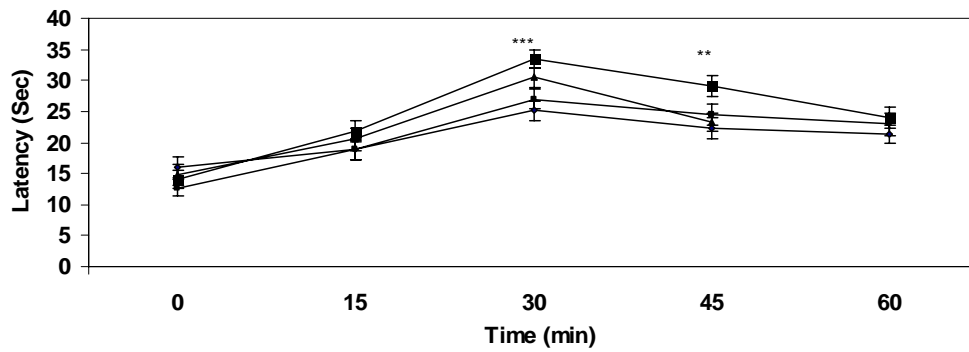


Figure 2. Effects of different doses of ketamine (●: 25, ▲: 50 and ■: 75 mg/kg, ip) on tolerance determined by hot plate test in morphine-tolerant mice. Each group had at least nine mice. Results are expressed as Mean ± SE. ** p<0.01, *** p<0.001, significantly different from the control group.

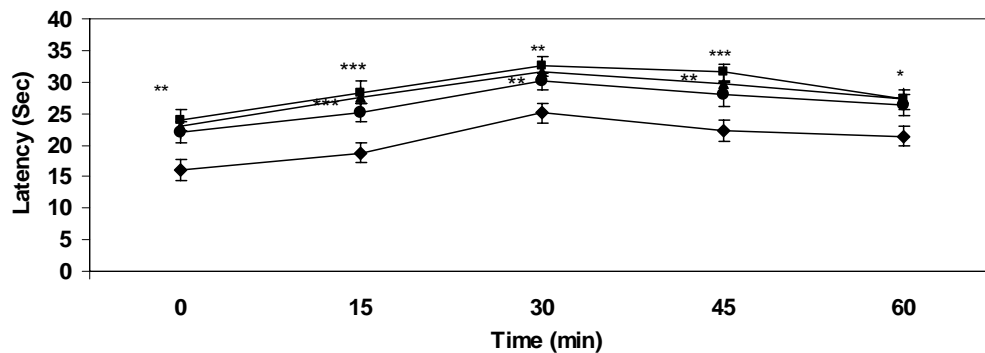


Figure 3. Effects of different doses of magnesium (●: 10, ▲: 20 and ■: 40 mg/kg, ip) on tolerance determined by hot plate test in morphine-tolerant mice. Each group had at least nine mice. Results are expressed as Mean ± SE. *<0.05, ** p<0.01, *** p<0.001, significantly different from the control group.

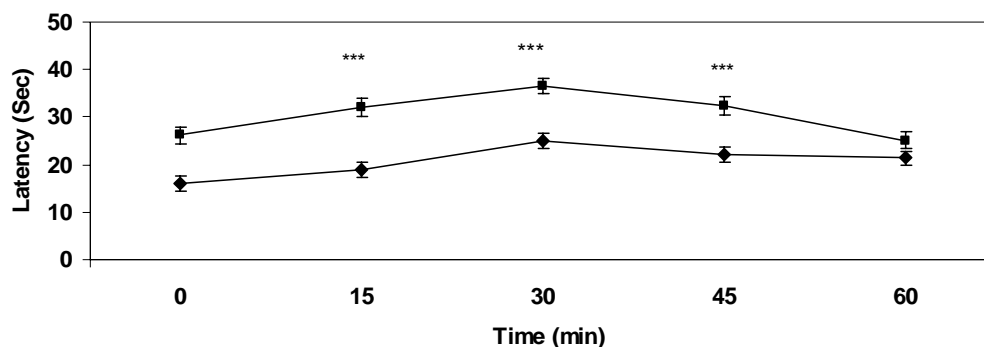


Figure 4. Effects of the use of ketamine (25mg/kg, ip) + magnesium (10 ml/kg, ip) on tolerance determined by hot plate test in morphine-tolerant mice. Each group had at least nine mice. Results are expressed as Mean \pm SE. *** $p < 0.001$, significantly different from the control group (\diamond)

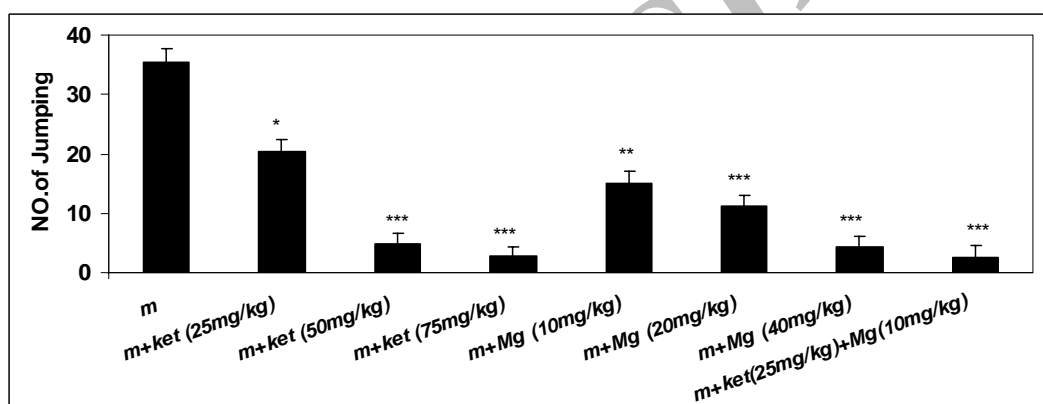


Figure 5. Effects of different doses of ketamine (25, 50, 75 mg/kg, ip) and magnesium (10, 20, 40 mg/kg, ip) and ketamine (50mg/kg, ip) + magnesium (1mg/kg, sc) on jumping induced by naloxone (4mg/kg, ip) in morphine dependent mice. Each group had at least nine mice. Results are expressed as Mean \pm SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from the morphine control group.

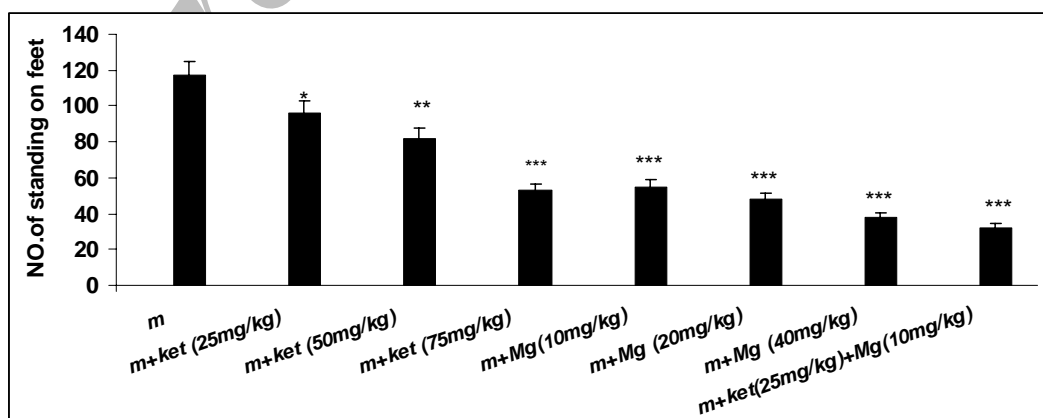


Figure 6. Effects of different doses of ketamine and magnesium and ketamine + magnesium on standing on feet induced by naloxone (4mg/kg, ip) in morphine dependent mice. Each group had at least nine mice. Results are expressed as Mean \pm SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from the morphine control group.

establishment of such long term changes (1-3,5-6). It has been shown that morphine withdrawal precipitates glutamate release (1-3). Conversely intra cerebroventricular glutamate or NMDA administration produces withdrawal signs in morphine-dependent rats (5-8,10). Excitatory synaptic input to ventral tegmental area (VTA) mediated by glutamate is a key component of the regulation of dopaminergic cells. The glutamate afferents arise from three primary sources: the medial prefrontal cortex, the pedunculopontine region and the subthalamic nucleus (20). Glutamate acts on AMPA, NMDA and mGluRs to depolarize dopamine neurons (21,22). Synaptically released glutamate may cause rapid and slow changes in the activity of dopaminergic cells. One role of glutamate innervation of the VTA is to mediation of a switch from pacemaker-like firing in dopaminergic cells to burst-firing pattern (23-25). Recent studies propose that repeated administration of opiate may activate the NMDA-receptor through G protein associated with opioid receptor and/or may have intracellular mechanisms (1-3,5). This opiate related activation of NMDA-receptors may initiate subsequent intracellular changes such as production of nitric oxide (NO) and/or activation of protein kinase C (PKC) (1-3). Both NO and PKC have been shown to be critical for development of morphine tolerance (1-3,5).

Previous studies (6,11-15) have shown that administration of ketamine attenuated intracellular Ca influx in both NMDA - receptor gated channel and voltage - gated Ca channel. The results of the present study show that ketamine (25, 50,75 mg/kg, ip) as NMDA- receptor antagonist, attenuates development of morphine tolerance

and dependence and withdrawal symptoms. Previous reports also indicated that ketamine was remarkably effective in reducing the incidences of withdrawal symptom in morphine dependent mice. The results indicate that NMDA mechanism(s) may be involved in the suppressive action of ketamine. On the other hand, long term administration of opiate leads to elimination of magnesium (Mg) blockade in the Ca channel and opening of the Ca channel of NMDA receptors and increase intracellular Ca.

Previous studies (16-18) have shown that administration of magnesium can attenuate the tolerance and dependence to the analgesic effects of morphine and its mechanism was related to the property of magnesium to block the Ca channel of NMDA receptors.

Different doses of magnesium (10, 20, 40 mg/kg, ip) which were employed in the present study also decreased tolerance and dependence to morphine. Additionally, present data indicates that a low dose of ketamine (25mg/kg, ip) in combination with a low dose of magnesium (10mg/kg, ip) decreased the development of morphine tolerance and dependence significantly ($P < 0.001$) which was evidenced by withdrawal symptoms. Ketamine seems to be a weak antagonist for NMDA receptor subtype. Ketamine induces emergence reactions and psychological manifestations. Therefore it will be ideal, if a low dose of ketamine (25mg/kg, ip) in combination with a low dose of magnesium (10mg/kg, ip) could attenuate morphine dependency and withdrawal symptoms) as well as development of morphine tolerance.

These results are related to the effect of this combination as antagonist of NMDA and NMDA Ca channel blocker.

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