

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

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ABSTRACT

The aim of this study was to investigate the effects of ketamine and midazolam on prevention of the development of morphine tolerance and dependence in mice. Different groups of mice received morphine (50 mg/kg, sc), morphine (50 mg/kg, sc) + ketamine (25,50,75 mg/kg, ip), morphine (50 mg/kg, sc) + midazolam (0.5,1,2 mg/kg, ip), morphine (50 mg/kg, sc) + ketamine (50 mg/kg, ip) + midazolam (1 mg/kg, ip) once a day for four days. Tolerance was assessed by administration of morphine (9 mg/kg, ip) on fifth day. Withdrawal symptoms were assessed by administration of naloxane (4 mg/kg, ip) two hours after co-administration of morphine with either ketamine or midazolam.

It was found that pretreatment with ketamine or midazolam decreased the degree of tolerance and withdrawal symptoms. Additionally co-administration of ketamine and midazolam before morphine therapy decreased the tolerance and dependence significantly. From these results it may be concluded that administration of ketamine and midazolam alone or in combination could prevent the development of tolerance and dependence to morphine. These effects can be related to the N-Methyl-D-Aspartate (NMDA) receptor antagonist behavior of ketamine and GABA-receptor agonist behavior of midazolam.

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

INTRODUCTION

It is known that continuous or long term use of opiate drugs may cause tolerance and dependence which limit the therapeutic efficacy of these drugs (1,2,3). Chronic opiate treatment leads to protein kinase C (PKC) activation and translocation (4,5,6) which phosphorylates the NMDA receptor-gated Ca channel, resulting in potentiation of NMDA receptor activity (1,3,5,6,7,8). 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 (1,2,3,5,6,9). On the other hand it is known that γ -Amino butyric acid (GABA) the major inhibitory neurotransmitter in the central nervous system (CNS) plays an important role in the development of tolerance and dependence in morphine therapy (10-13).

Midazolam as a benzodiazepine-receptor agonist, has been widely used for induction and maintenance of anesthesia with opioids or inhaled anesthetic in clinics (10, 11,13). This medication suppresses withdrawal responses by inhibition of the hyper sensitization of the spinal cord nervous (11). Midazolam may occupy the benzodiazepine receptor on the benzodiazepine GABA-Cl channel complex and therefore

facilitates the inhibitory action of GABA on neuronal transmission. Midazolam could prolong the antinociceptive effect of morphine by delaying in the chronic morphine-induced development of tolerance to antinociception in rats (10,11,13). In the present investigation the possible interaction between opiate and NMDA and GABA receptors regarding tolerance and withdrawal were studied.

MATERIALS AND METHOD

Animals: Male mice (20-30g) were used in this study. Pain sensitivity was measured by hot-plate test.

Drugs: Morphine sulfate (Darupakhsh, Iran), ketamine hydrochloride (Rotexmedica, Germany), Midazolam hydrochloride (Dormicum, Canada), Naloxane Hydrochloride (Tolidaru, Iran)

Methods

Hot-plate test

Each animal was placed on a surface (23 × 23 Cm) maintained at 55 ± 2 °C surrounded by a Plexiglas wall 20 Cm high. Licking of hands was used at the end point for determination of response latencies. Failure to respond by 45 seconds was a marker for 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) by morphine (50 mg/kg) in combination with either ketamine or midazolam or both once a day for four days. To evaluate the degree of tolerance, the antinociceptive effect of a test dose of morphine (9 mg/kg) was measured 24 hours after the last dose of morphine in combination with ketamine or midazolam or both.

Induction of dependence

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

Evaluation of the withdrawal syndrome

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

Statistical Analysis

The results are expressed as the Mean±SE. Differences between the individual mean values in different groups were analyzed by one-way analysis of variance (ANOVA) 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). Animals that became tolerant to effects of morphine exhibited only a small antinociceptive effect (Fig.1 and 2).

Naloxane-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 naloxane was chosen for induction of withdrawal symptoms. Naloxane induced withdrawal signs: jumping and standing on feet (Fig.6 and 7).

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

As shown in figure.3, ketamine injection (25,50,75 mg/kg, ip) 30 min before daily morphine administration, decreased tolerance to the analgesic effects of morphine significantly. Figures. 6 and 7 show that pretreatment with ketamine (25,50,75 mg/kg, ip) dose dependently decreased the withdrawal symptoms significantly.

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

As shown in figure.4, injection of midazolam (0.5,1,2 mg/kg, ip) 30 min before daily morphine administration decreased tolerance to the analgesic effect of morphine significantly. Figures.6 and 7 show that pretreatment with midazolam (0.5,1,2 mg/kg, ip) dose dependently decreased the withdrawal symptoms significantly.

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

As shown in Figure.5, co-administration of ketamine (50 mg/kg, ip) and midazolam (1 mg/kg, ip) 30 min before daily morphine administration decreased tolerance phenomenon significantly and figures 6 and 7 show that this combination decreased withdrawal symptoms significantly.

DISCUSSION

The principle aim of this study was to evaluate the effects of ketamine (as a non competitive NMDA receptor antagonist) and midazolam (as a benzodiazepine receptor agonist) on development of tolerance and withdrawal symptoms. It has been proposed that repeated administration of opiate may activate the NMDA - receptor through G protein associated with opioid receptor and / or intracellular mechanisms (4-7)

This opiate related activation of NMDA-receptors may initiate subsequent intracellular changes such as production of nitric oxide (NO) and / or the activation of protein kinase C (PKC) (5-7). Both NO and PKC have been shown to be critical for development of morphine tolerance (5,6). The results of the present study show that the NMDA-receptor antagonists such as ketamine (25, 50,75 mg/kg, ip) may attenuates development of morphine tolerance and dependence and withdrawal symptoms.

Previous studies (2,6,7) have shown that administration of ketamine attenuated intracellular Ca²⁺ influx both in NMDA-receptor gated channel as well as in voltage-gated Ca channel.

Other studies have shown that there is an interaction between GABA and opioid system and GABAergic system has a role in opioid tolerance and dependence (10-13). Both GABA_A- and GABA_B-mediated synaptic potentials in dopaminergic cells of the ventral tegmental area (VTA) were inhibited presynaptically by opioids. The GABA_A-mediated synaptic potential is thought to arise from inter neurons that are hyperpolarized by opioids (14). The inhibition of spontaneous activity recorded from inter neurons correlated with the inhibition of tetrodotoxin-

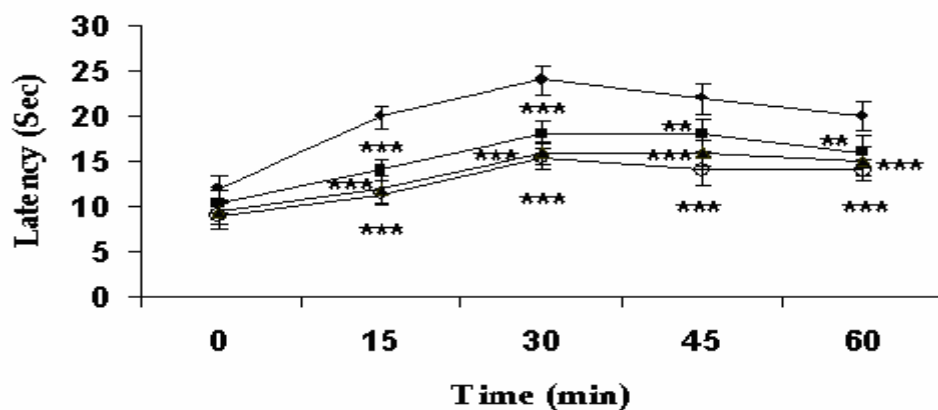


Fig.1 Effects of morphine on tolerant and non tolerant mice. Animals were injected morphine (50 mg/kg, sc) for 2 (■), 3 (▲) or 4 (×) days. Antinociception of a test dose of morphine (9 mg/kg, sc) was assayed 24 hour after the last dose of morphine (50 mg/kg, sc). Each group had at least 9 mice. Results are expressed as Mean±SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from the respective non tolerant control group (day 1).

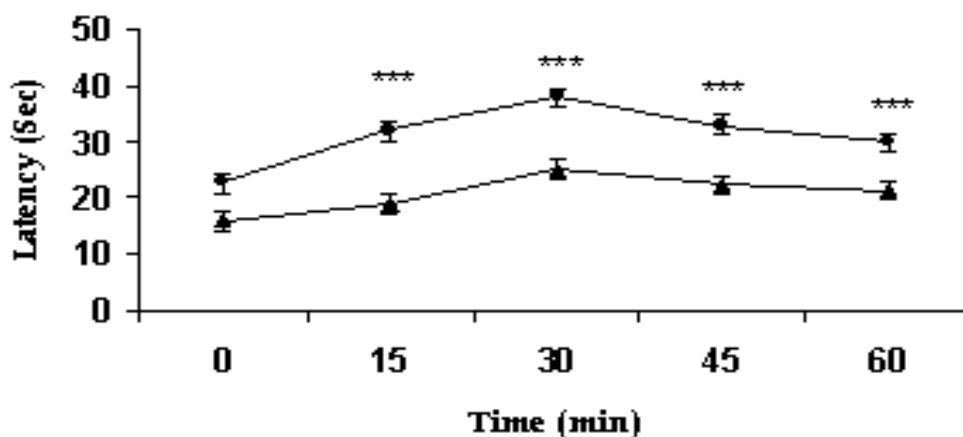


Fig.2. Effects of morphine on tolerant and non tolerant mice. Animals received either saline (10 ml/kg, sc) or morphine (50 mg/kg, Sc) +saline (10 ml/kg, sc) for 4 days. Antinociception of a test dose of morphine (9 mg/kg, sc) was tested 24 hour after the last dose of morphine (50 mg/kg, sc) in tolerant and non tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from the respective non tolerant control group.

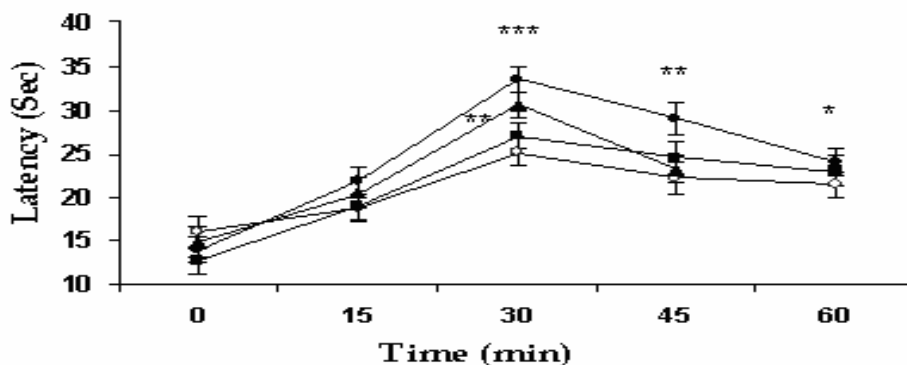


Fig.3. Effects of different doses of ketamine (25,50,75 mg/kg, ip) on tolerance determined by hot-plate test in morphine-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, significantly different from the control group.

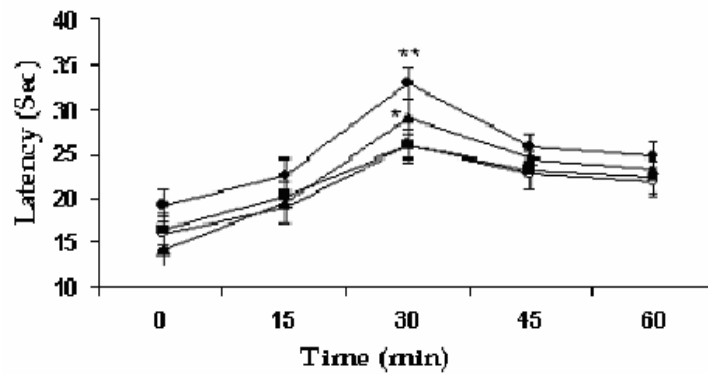


Fig.4. Effects of different doses of midazolam (0.5,1,2 mg/kg, ip) on tolerance determined by hot-plate test in morphine-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE. *p<0.05 , **p<0.01 , ***p<0.001, significantly different from the control group.

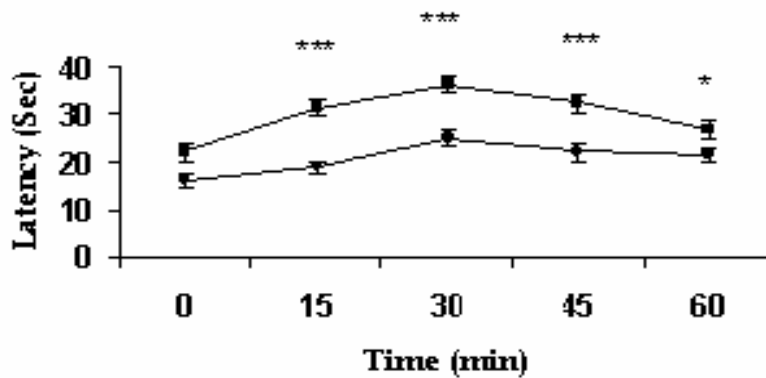


Fig.5. Effects of the use of ketamine (50 mg/kg, ip) + midazolam (1 mg/kg, ip) on tolerance determined by hot-plate test in morphine-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE. * p<0.05 , ** p<0.01 , ***p<0.001, significantly different from the control group.

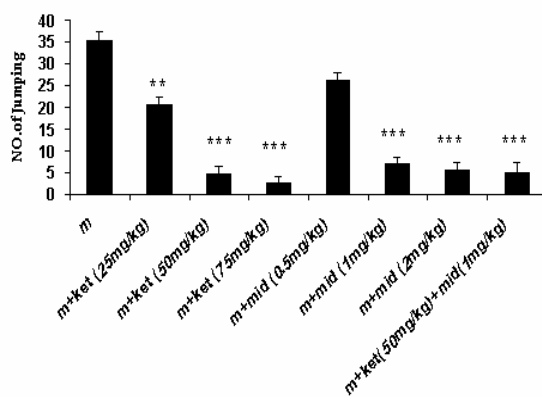


Fig.6. Effects of different doses of ketamine (25,50,75 mg/kg, ip) and midazolam (0.5,1,2 mg/kg, ip) and ketamine (50 mg/kg, ip)+ midazolam (1 mg/kg, ip) on jumping induced by naloxane (4 mg/kg, ip) in morphine-dependent mice. Each group had at least 9 mice. Results are expressed as Mean±SE. * p<0.05 , ** p<0.01 , ***p<0.001, significantly different from the morphine control group.

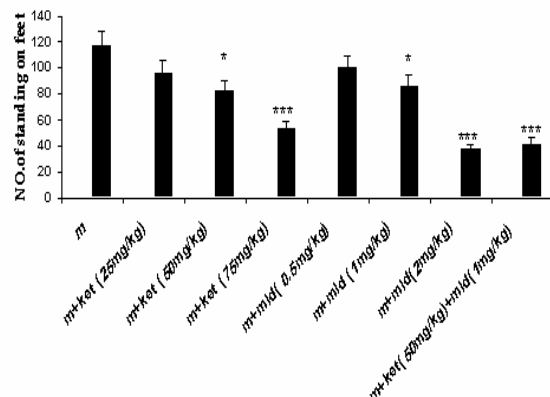


Fig.7. Effects of different doses of ketamine and midazolam and ketamine+ midazolam on standing on feet induced by naloxane (4 mg/kg, ip) in morphine-dependent mice. Each group had at least 9 mice. Results are expressed as Mean±SE . * p<0.05 , ** p<0.01 , ***p<0.001, significantly different from the morphine group.

sensitive GABA-mediated IPSPs recorded in dopaminergic cells (14). It has been concluded that cells that were hyperpolarized by μ -opioid receptors in the VTA were GABA interneurons.

The GABA_B IPSP is thought to arise from fibers originating in the nucleus accumbens or ventral pallidum. On the basis of selective effects of 5-HT and dopamine on GABA_A and GABA_B IPSPs, separate terminals are thought to mediate these synaptic responses (12,15). The GABA_B IPSP was increased by D₁-dopamine agonists and decreased by 5-HT_{1B} agonists, whereas the GABA_A IPSP was insensitive to both agonists. Unlike inhibition of the GABA_A IPSP, both μ - and κ -opioid agonists decreased the GABA_B IPSP by a presynaptic mechanism (14,16). Thus the activation of opioid receptors on at least two types of GABA-releasing terminals decrease GABA-

mediated inhibition, allowing an increase in activity through disinhibition (14,17). In the present study pretreatment with midazolam (0.5,1,2 mg/kg, ip) 30 min before daily morphine administration may prevent the development of morphine tolerance and dependence.

Furthermore co-administration of ketamine and midazolam have shown that the use of this combination decrease development of morphine tolerance and dependence and withdrawal symptoms significantly. On the other hand other studies have shown that NMDA-receptor antagonists such as ketamine or phencyclidine have some psychotic adverse effects which are treated by benzodiazepine which suggests that the combination of midazolam and ketamine might be useful for elimination of ketamine adverse effects.

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