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N-methyl-D-aspartate receptors involved in morphine-induced hyperalgesia in sensitized mice

Shamseddin Ahmadi [a,*], Hajar Golbaghi [b], Ronak Azizbeigi [c], Nabaz Esmailzadeh [d]

[a] Department of Biological Science and Biotechnology, Faculty of Science, University of Kurdistan, P.O. Box 66167-15145, Sanandaj, Iran
[b] Department of Biology, Faculty of Science, Islamic Azad University, Hamedan Branch, Hamedan, Iran
[c] Department of Physiology, Faculty of Veterinary Science, Islamic Azad University, Sanandaj Branch, Sanandaj, Iran
[d] Department of Statistics, Faculty of Science, University of Kurdistan, Sanandaj, Iran

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ABSTRACT

The aim of this study was to investigate role of the N-Methyl-D-Aspartate (NMDA) receptors in the decrease of morphine analgesia in mice after nociceptive sensitization. We used a hot plate test to assess effects of morphine on pain behavior in male NMRI mice. All drugs were administered through an intraperitoneal route. Sensitization schedule composed of 3-days pre-treatment of morphine (20 mg/kg) followed by 5-days washout. The results showed that morphine (5, 7.5, 10 and 15 mg/kg) induced a significant analgesia in normal mice. However, the analgesic effects of morphine significantly decreased at higher dose (15 mg/kg) in sensitized mice. Injections of either a competitive NMDA receptor antagonist, D-AP5 (0, 0.25, 0.5 and 1 mg/kg) or an NMDA receptor channel blocker (30, 60 and 120 mg/kg) alone had no effect on pain behavior. However, injections of D-AP5 (1 mg/kg), along with morphine over 3-days of the sensitization schedule, significantly prevented the decrease in the analgesic effect of the opioid at doses of 7.5 and 10 mg/kg on the hot plate test. Similarly, injections of MgSO4 (120 mg/kg), along with morphine over 3-days of the sensitization schedule, significantly prevented the decrease in analgesic effect of morphine at doses of 10 and 15 mg/kg. It can be concluded that NMDA receptors are influenced by morphine during the sensitization schedule, which in turn may affect morphine analgesia after the schedule. This may further support the potential effectiveness of NMDA blockade during repeated use of morphine for control of chronic pain.

1. Introduction

Opioid analgesics are still the most effective and frequently used pain relievers (Benyamin et al., 2008; Cunha et al., 2010; Somogyi et al., 2007). However, other than the common side effects associated with opioid analgesics, continuous morphine therapy has been shown to induce hyperalgesia (Chu et al., 2008). According to research, a state of nociceptive sensitization may be caused by exposure to opioids leading to opioid-induced hyperalgesia, which is believed to be different from tolerance in some aspects (Lee et al., 2011; Silverman, 2009). Since mechanisms behind this phenomenon remain elusive, study on associated neurochemical changes and mechanisms still need to be continued.

N-Methyl-D-Aspartate (NMDA) receptors have shown to be involved in expression of morphine tolerance and dependence (Mori and Mishina, 1995). Gudehithlu et al. (1994) were the first to report that long-term treatment with morphine caused change of NMDA receptors in the rat brain (Gudehithlu et al., 1994). Later, it was found that the expression of NMDA receptors became upregulated in the brain of morphine-dependent rats (Koyuncuoglu et al., 1999). Mao (1999) also reported that intraperitoneal or intrathecal administrations of NMDA receptor antagonists during chronic morphine treatments resulted in the inhibition of morphine tolerance and dependence [for review see (Mao, 1999)]. This was in line with a later report by Bisaga et al. (2001) which indicated that glutamatergic signal transduction, mediated by NMDA receptors, were involved in the formation and maintenance of morphine dependence in humans (Bisaga et al., 2001).

Furthermore, NMDA subtype of glutamate receptors, located pre- and post-synaptically on dorsal horn neurons of the spinal cord, have a pivotal role in transmission of pain signals (Willcockson and Valtschanoff, 2008). It has been shown that NMDA receptors play an important role in more prolonged pain states to enhance, prolong and alter activity in nociceptive circuitry in the spinal cord where it seems to be responsible for hyperalgesia (Dickenson, 1997; Marvizon et al., 2002). According to the research, NMDA receptors antagonism by D-AP5 effectively attenuated analgesic tolerance to morphine

* Corresponding author. Tel.: +98 871 6660075; fax: +98 871 6622702.
E-mail addresses: sh.ahmadi@uok.ac.ir, shamseddin2000@yahoo.com (S. Ahmadi).

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(Bilsky et al., 1996; Wong et al., 1996). NMDA receptor antagonists appear to inhibit the neural plasticity underlying some forms of opiate tolerance, sensitization and physical dependence suggesting that NMDA receptors are involved in the development of changes in behavior induced by opioids (Trujillo, 2000). However, using NMDA antagonists may be limited because of their side effects of, for example, neuronal damage (Horvath et al., 1997). Magnesium ions (Mg^{2+}) are also NMDA receptor channel blockers at normal concentrations (Nikolaev et al., 2012). Some reports have shown that Mg^{2+} may cause antinociceptive effects by itself and along with morphine in different models of pain assessment (Begon et al., 2002; Bujalska et al., 2008; Kroin et al., 2000). Therefore, the possibility investigated in the present study was whether NMDA receptors blockade by D-AP5 or Mg^{2+} ions underlie adaptive changes due to sensitization to morphine and subsequent hyperalgesia induced by the opioid.

2. Materials and methods

2.1. Animals

Three hundred and forty adult male albino NMRI mice weighing 20–30 g (Pasteur Institute, Tehran, Iran) were used. They were kept in an animal house with a 12/12-h light/dark cycle (light on at 7:00 a.m.) and controlled temperature (22 ± 2 °C). The animals were housed in groups of 10 in plexiglas cages with free access to food and water. Behavioral tests were performed during the light phase of the cycle, and each animal was tested once only. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (2011), prepared by the National Academy of Sciences' Institute for Laboratory Animal Research.

2.2. Drugs

Morphine sulfate, an opioid agonist, was purchased from Temad (Tehran, Iran); D-(-)-2-Amino-5-phosphonopentanoic acid (D-AP5), a competitive NMDA receptor antagonist, was purchased from Ascent Scientific (Bristol, UK); and magnesium sulfate ($MgSO_4$), an NMDA receptor channel blocker, was a gift from Merck (Germany). All drugs were dissolved in saline (0.9% solution) before each use, and injected through an intraperitoneal route at a volume of 10 ml/kg. The drug doses used in this study were based either on a pilot study or the previous literature data (Bujalska et al., 2009; Ulugol et al., 2002).

2.3. Hot plate test of analgesia

A hot plate test was used to assess pain behavior. On the day of testing, mice were acclimated to the testing environment for 30 min, then each animal placed in a glass square on a hot plate apparatus (Armaghan Co., Iran), with a set temperature of 55 ± 0.1 °C. The time between the placements of each animal on the hot plate till they either licked their hind paws or first jump was recorded as a nociceptive response. A cutoff time of 80 s was defined as complete analgesia. First, the hot plate test was performed for each animal to access "baseline latency" prior to treatments. Second, mice were received saline or morphine treatments and 30 min later tested for "test latency". Finally, the recorded latencies were converted to percentage maximum possible effect (%MPE) based on to the following formula: %MPE = [(test latency − baseline latency)/(cut-off time − baseline latency)] × 100 (Keil and Delander, 1995; Ossipov et al., 1990).

2.4. Induction of nociceptive sensitization with pre-treatment of morphine in mice

A schedule of sensitization was used for eight days. First, a dose of 20 mg/kg of morphine was daily injected intraperitoneally for three consecutive days followed by five days of no drug treatment (washout). The control mice underwent a similar schedule but they received normal saline instead of morphine. On day 9 (one day after the schedule) the animals were tested for pain behavior on the hot plate apparatus.

2.5. Experimental design

2.5.1. Experiment 1: antinociceptive effect of morphine in normal and sensitized mice

To examine induction of nociceptive sensitization by morphine, one group of animals received morphine (20 mg/kg) for three days and each day, 30 min after morphine injection, hotplate test was performed to assess pain behavior. Then, on day 9 (one day after 5-days washout) hotplate test was also carried out to assess effects of morphine (20 mg/kg) on pain behavior. Ten other groups of animals were used in this experiment. Five groups received saline for three days followed by five days of no drug treatment and then on the test day (day 9) they received saline or different doses of morphine (5, 7.5, 10 and 15 mg/kg) 30 min before testing. The other five groups of the animals received morphine (20 mg/kg) for three days followed by five days drug free, and on the test day (day 9) they received saline or different doses of morphine (5, 7.5, 10 and 15 mg/kg) 30 min before the hot plate test.

2.5.2. Experiment 2: effects of combination treatments of D-AP5 and morphine during 3-days followed by 5 days wash out on pain behavior in mice

First, we examined effects of different doses of D-AP5 (0, 0.25, 0.5 and 1 mg/kg) by itself on pain behavior. Second, ten groups of mice were divided into two sets of normal and sensitized mice. Five groups of the normal mice received morphine (20 mg/kg) plus saline, but the groups of the sensitized mice received morphine plus D-AP5 (1 mg/kg) during 3-days of the sensitization schedule. On the test day, the groups of both sets received saline or morphine at the different doses (5, 7.5, 10 and 15 mg/kg), 30 min before the hot plate test.

2.5.3. Experiment 3: effects of combination treatments of $MgSO_4$ along with morphine during 3-days followed by 5 days wash out on pain behavior in mice

First, we examined effects of different doses of $MgSO_4$ (0, 30, 60 and 120 mg/kg) by itself on pain behavior. Five groups of normal mice used in experiment 2 which received morphine (20 mg/kg) plus saline during 3-days of the sensitization schedule were also used as control groups in this experiment. Five other groups of sensitized mice received morphine plus $MgSO_4$ (120 mg/kg) during 3-days of morphine sensitization. On the test day, they received saline or morphine at different doses (5, 7.5, 10 and 15 mg/kg), 30 min before the hot plate test.

2.6. Statistical analysis

All data were presented as mean ± S.E.M. of %MPE related to ten animals in each experimental group. The results of Shapiro–Wilk test for normality of data revealed that data was normal ($P > 0.05$). One-way repeated measure ANOVA, one-way ANOVA, and two-way ANOVA were used for analyzing data where appropriate. Following a significant F-value, post-hoc Tukey's test was performed to assess paired groups comparisons. $P < 0.05$ was