

ATP-sensitive Potassium Channels and L-type Calcium Channels are Involved in Morphine-induced Hyperalgesia after Nociceptive Sensitization in Mice

Shamseddin Ahmadi^{1*}, Shaho Azarian², Sayede Shohre Ebrahimi¹, Ameneh Rezayof²

1. Department of Biological Science and Biotechnology, Faculty of Science, University of Kurdistan, Sanandaj, Iran.

2. Department of Animal Biology, School of Biology, University college of Science, University of Tehran, Tehran, Iran.

Article info:

Received: 31 December 2013

First Revision: 07 February 2014

Accepted: 11 February 2014

Key Words:

Pain Behavior,
Sensitization,
Morphine,
Diazoxide,
Nimodipine.

ABSTRACT

Introduction: We investigated the role of ATP-sensitive potassium channels and L-type calcium channels in morphine-induced hyperalgesia after nociceptive sensitization.

Methods: We used a hotplate apparatus to assess pain behavior in male NMRI mice. Nociceptive sensitization was induced by three days injection of morphine and five days of drug free. On day 9 of the schedule, pain behavior test was performed for evaluating the effects of morphine by itself and along with nimodipine, a blocker of L-type calcium channels and diazoxide, an opener of ATP-sensitive potassium channels. All drugs were injected through an intraperitoneal route.

Results: The results showed that morphine (7.5, 10 and 15 mg/kg) induced analgesia in normal mice, which was prevented by naloxone (1 mg/kg). After nociceptive sensitization, analgesic effect of morphine (10 and 15 mg/kg) was significantly decreased in sensitized mice. The results showed that nimodipine (2.5, 5, 10 and 20 mg/kg) had no significant effect on pain behavior test in either normal or sensitized mice. However, nimodipine (20 mg/kg) along with morphine (10 and 15 mg/kg) caused more decrease in morphine analgesia in sensitized mice. Furthermore, diazoxide by itself (0.25, 1, 5 and 20 mg/kg) had also no significant effect on pain behavior in both normal and sensitized mice, but at dose of 20 mg/kg along with morphine (10 and 15 mg/kg) decreased analgesic effect of morphine in sensitized mice.

Discussion: It can be concluded that potassium and calcium channels have some roles in decrease of analgesic effect of morphine after nociceptive sensitization induced by pretreatment of morphine.

1. Introduction

Morphine is among the most effective and commonly used analgesics for controlling moderate to severe pain (Benyamin et al., 2008; Cunha et al., 2010; Somogyi, Barratt & Collier, 2007). It has been shown that antinociceptive effect of morphine is mediated via an inhibitory G protein, which inhibits cAMP formation and calcium (Ca²⁺) conduc-

tance, while activates potassium (K⁺) conductance that induces hyperpolarization of nociceptive cells (Nestler, 2004; Rodrigues & Duarte, 2000). Therefore, although morphine effects are mediated by mu-opioid receptors but ion channels may play important roles in its effects. In support of this idea, some evidence has indicated that ATP-sensitive potassium (K⁺ATP) channels are involved in morphine analgesia (Chiou & How, 2001). It has been also shown that morphine, via activation of a signaling pathway including K⁺ATP channels, blocks

* Corresponding Author:

Shamseddin Ahmadi, PhD

Department of Biological Science and Biotechnology, Faculty of Science, University of Kurdistan, P.O. Box 416, Sanandaj, Iran.

Tel: +98(871)6660075 / Fax: +98(871)6622702

E-mail: sh.ahmadi@uok.ac.ir

hypernociception by changes in membrane potential of nociceptive neurons (Cunha et al., 2010). Furthermore, blockade of calcium channels has been also reported to alter analgesic effects of morphine in laboratory animals. In particular, it has been shown that both central and systemic administrations of L-type calcium channel blockers potentiate morphine analgesia (Benedek & Szikszay, 1984; Contreras, Tamayo & Amigo, 1988; Del Pozo, Caro & Baeyens, 1987; Dogrul, Yesilyurt, Isimer & Guzeldemir, 2001; Omote, Sonoda, Kawamata, Iwasaki & Namiki, 1993).

Besides its well-known antinociceptive actions, morphine can cause hyperalgesia by an unknown opioid receptor-independent mechanism (Chu, Angst & Clark, 2008). Opioid-induced hyperalgesia is defined as a state of nociceptive sensitization caused by exposure to opioids, which is related to but different from tolerance (Lee, Silverman, Hansen, Patel & Manchikanti, 2011; Silverman, 2009). Although this effect is constantly reported across literature, the neurochemical changes and mechanisms associated with this phenomenon remain unknown (Chu, Angst & Clark, 2008). Adaptive change in neurons is a hallmark of chronic morphine treatment, and related to altered behaviors associated with morphine dependence (DuPen, Shen & Ersek, 2007). However, the molecular and cellular mechanisms underlying these long-lasting changes are not still fully understood (Lee, Silverman, Hansen, Patel & Manchikanti, 2011; Raffa & Pergolizzi, 2012).

It has been reported that a regimen of 3 days morphine and then 5 days washout may induce sensitization to morphine (Rezayof, Assadpour & Alijanpour, 2013; Zarindast & Rezayof, 2004). We have recently shown that blockades of K⁺ ATP and Ca²⁺ channels decrease the analgesic effect of morphine in diabetic mice (Ahmadi, Ebrahimi, Oryan & Rafeenia, 2013). According to research, a sensitization process in pain pathways may be involved in diabetic-induced hyperalgesia (Kamei et al., 1994; Voitenko, Kruglikov, Kostyuk & Kostyuk, 2000). Considering these backgrounds, the aim of the present study was to investigate the role of K⁺ ATP and Ca²⁺ channels in the decrease of analgesic effect of morphine in mice after nociceptive sensitization induced by morphine.

2. Methods

2.1. Subjects

Adult male albino NMRI mice weighing 20-30 g (Pasteur institute, Tehran, Iran) 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). They were housed in groups of 10 in Plexiglas cages with free access to food and water. Pain behavior test was performed during the light phase of the cycle, and each animal was tested once only. All procedures were performed in accordance with guide of National Academy of Sciences' Institute (NASI) for care and use of laboratory animals (2011).

2.2. Drugs

Morphine sulfate was purchased from Temad (Tehran, Iran). Naloxone hydrochloride, diazoxide, an K⁺ ATP opener, and nimodipine, a blocker of L-type and Ca²⁺ channels were purchased from Ascent Scientific (Bristol, UK). Morphine and naloxone were dissolved in saline (0.9 %, w/v solution) before each use, while nimodipine and diazoxide were dissolved in a vehicle composed of dimethylsulfoxide (DMSO) and saline (1:1 v/v solution). All drugs were injected through an intraperitoneal route at a volume of 10 ml/kg. Drug doses were selected either from pilot experiments or other studies (Biala & Weglinska, 2006; Sukriti, Hota & Pandhi, 2004).

2.3. Induction of Nociceptive Sensitization in Mice

Nociceptive sensitization schedule was performed during 8 days. First, mice were injected intraperitoneally with morphine (20 mg/kg) for three consecutive days, and then they were allowed to spend five days of drug free (wash out). Control group only received normal saline instead of morphine in the same way. One day after the sensitization schedule (on day 9), the animals were tested for pain behavior on a hotplate apparatus.

2.4. Hotplate Test

A hotplate apparatus (Armaghan Co., Iran), was used to assess algesic or analgesic effects of drugs. On the testing day, the mice were acclimated to the testing environment for 30 min, and then each animal placed on the plate of apparatus which its temperature was set at 55±0.1°C. A glass square (height 25 cm) was placed on the hotplate to prevent escaping of the animal. Pain behavior was defined as either licking of the hind paws or first jumping of the animal. The time elapse from the placement of the animal on the hotplate until observing pain behavior was recorded as baseline or test latencies. A cutoff time of 120 s was defined to avoid tissue damage. "Baseline latency" was measured just prior to drug administration, and "test latency" was recorded after drug treatments at time intervals of 30 min after morphine, 20 min after naloxone or 45 min after nimodipine and diazoxide. Finally, the recorded "baseline latency" and

“test latency” were converted to percent maximum possible effect (%MPE) according to the following formula: $\%MPE = [(test\ latency - baseline\ latency) / (cut-off\ time - baseline\ latency)] \times 100$.

2.5. Experimental Design

2.5.1. Experiment 1: Effects of Morphine on Pain Behavior Test in Normal and Sensitized Mice

Fifteen groups of animals were used. Ten groups of them received saline during three days followed by five days washout. On hotplate test day, baseline latency was recorded for each animal. Then, test latency was recorded for five groups of these animals 30 min after administrations of saline or morphine (5, 7.5, 10 and 15 mg/kg). The other five groups received saline or morphine (5, 7.5, 10 and 15 mg/kg) at 30 min before testing plus naloxone at 20 min before testing, then test latency was recorded. The last five groups of the animals received pretreatment of morphine (20 mg/kg) for three days followed by five days wash out to induce nociceptive sensitization. On the hotplate test day (day 9), baseline latency was firstly recorded, and then test latency was measured at 30 min after administrations of saline or morphine (5, 7.5, 10 and 15 mg/kg).

2.5.2. Experiment 2: Effects of Nimodipine by itself on Pain Behavior Test in Normal and Sensitized Mice

In this experiment, ten groups of mice were used. Five groups of them received pretreatment of morphine (20 mg/kg) for three days, and then they were allowed to spend five days wash out to induce nociceptive sensitization. The other five groups as normal mice received saline instead of morphine during sensitization schedule. On hotplate test day, baseline latency was firstly recorded for each animal, then five groups of either normal or sensitized mice received saline or nimodipine (2.5, 5, 10 and 20 mg/kg), and test latency was recorded 45 min after administrations of the drugs.

2.5.3. Experiment 3: Effects of Nimodipine along with Morphine on Pain Behavior Test in Sensitized Mice

Ten groups of animals were submitted to the nociceptive sensitization schedule. On the hotplate test day, baseline latency was measured for each animal. Then, five groups of them received vehicle (10 ml/kg) but the other five groups received nimodipine (20 mg/kg) at 45 min before recording the test latency. Fifteen min after vehicle or nimodipine injections, five groups of both sets

received saline or different doses of morphine (5, 7.5, 10 and 15 mg/kg), and test latency was measured 30 min after the last injection.

2.5.4. Experiment 4: Effects of Diazoxide by itself on Pain Behavior Test in Normal and Sensitized Mice

Ten groups of mice were used. Five groups of them received pretreatment of morphine (20 mg/kg) for three days followed by five days wash out to induce nociceptive sensitization, but the other five groups as normal mice only received saline. On hotplate test day, baseline latency was firstly recorded, then five groups of either normal or sensitized mice received saline or diazoxide (0.25, 1, 5 and 20 mg/kg), and test latency was recorded 45 min after administrations of the drugs.

2.5.5. Experiment 5: Effects of Diazoxide along with Morphine on Pain Behavior Test in Sensitized Mice

In this experiment, five groups of animals were submitted to nociceptive sensitization schedule. On the hotplate test day, baseline latency was firstly recorded. Then, all groups immediately received diazoxide (20 mg/kg), 15 min later, they received saline or morphine (5, 7.5, 10 and 15 mg/kg), and 30 min after the last injection, the test latency was recorded for each animal. Five groups of mice as control groups of experiment 3, which received vehicle (instead of diazoxide) plus saline or morphine (5, 7.5, 10 and 15 mg/kg) were also considered as control groups for experiment 5.

2.6. Statistical Analysis

All data were presented as mean±S.E.M. of %MPE related to ten animals in each group. One- or two-way analysis of variance (ANOVA) was used for analyzing data. Following a significant F-value, post-hoc t-test was performed to assess paired groups comparisons. $P < 0.05$ was considered as statistically significant level.

3. Results

3.1. Morphine Induced Analgesia in Normal Mice, but its Analgesic Effect was Decreased in Sensitized Mice

The results of experiment 1 showed that morphine induced significant analgesia in normal mice [one-way ANOVA, $F(4, 45) = 21.74$, $P < 0.001$]. Post-hoc t-test revealed that morphine at doses of 7.5, 10 and 15 mg/kg induced significant analgesia compared to saline-treated

control group in normal mice. One-way ANOVA also revealed that injections of naloxone along with morphine decreased analgesic effect of the later drug, as revealed by decrease of between-group significance [one-way ANOVA, $F(4, 45) = 3.37, P < 0.05$]. Posthoc t-test revealed that naloxone almost completely prevented the analgesic effect of different doses of morphine except for 15 mg/kg. Two-way ANOVA showed a significant interaction between sensitization schedule (as factor A with two levels) and morphine treatment on hotplate test (as factor B with five levels) [$F(4, 90) = 3.29, P < 0.05$]. However, although sensitization induced a significant decrease in the opioid analgesia compared to normal mice, but its effect was not equal to the blockade of opioid receptors by naloxone (Fig. 1).

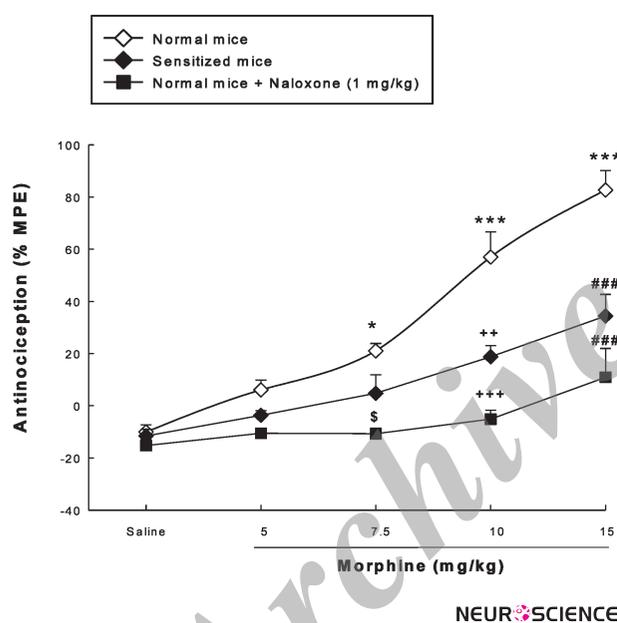
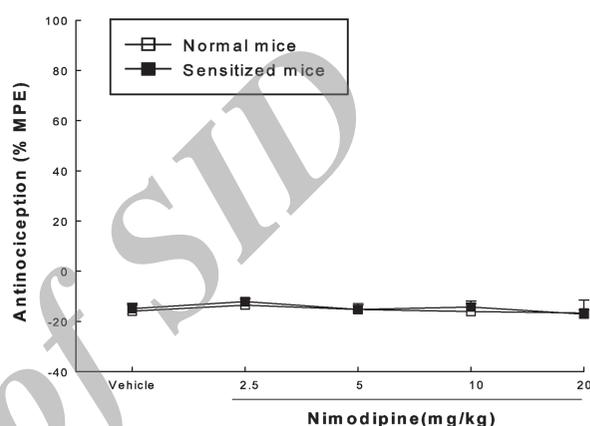


Figure 1. Effects of morphine on pain behavior test in normal and sensitized mice. Ten groups of animals ($n=10$) received saline for 3 days, and then were allowed for 5-days drug free. Then, on the test day (day 9) five groups of them (points shown by white square symbol) received saline or different doses of morphine (5, 7.5, 10 and 15 mg/kg), 30 min before examining test latency, while the other five groups of them (points indicated by black square symbol) received the same treatment plus naloxone. Five groups ($n=10$) of animals (points indicated by black triangle symbol) also received morphine (20 mg/kg) during 3-days nociceptive sensitization schedule, and then were allowed for 5-days drug free. 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 examining test latency. Each point is the mean \pm S.E.M. of %MPE. * $P < 0.05$ and *** $P < 0.001$ compared to saline control group in normal mice. ++ $P < 0.01$ and ### $P < 0.001$ compared to the group that received the same dose of morphine in the normal mice.

3.2. Nimodipine by itself had neither Algesic nor Analgesic Effects in both Normal and Sensitized Mice

One-way ANOVA revealed that nimodipine injection on hotplate test day caused no change in pain behavior test of either normal mice [one-way ANOVA, $F(4, 45) = 0.12, P > 0.05$], or sensitized mice [one-way ANOVA, $F(4, 45) = 1.06, P > 0.05$] (Fig. 2).

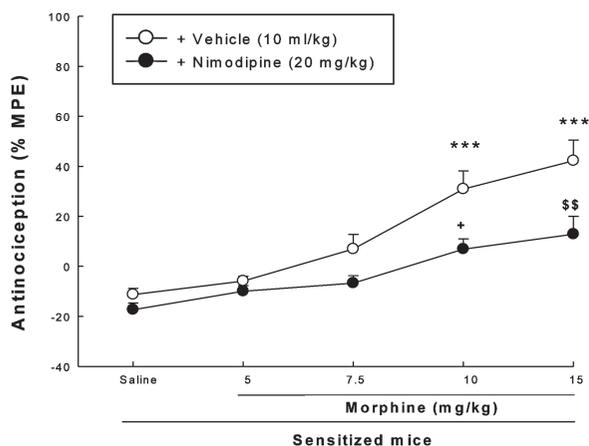


NEURSCIENCE

Figure 2. Effects of nimodipine by itself on pain behavior test in normal and sensitized mice. Ten groups of animals ($n=10$) were used, five groups as normal (points shown by white square symbol) and the other five groups as sensitized mice (points symbolized by black triangle). Each five groups of the animals on hotplate test day received either vehicle (10 ml/kg) or nimodipine (2.5, 5, 10 and 20 mg/kg), 45 min before testing. Each point is the mean \pm S.E.M. of %MPE.

3.3. Nimodipine Decreased Analgesic Effect of Morphine in Sensitized Mice

A two-way ANOVA was conducted to explore the effect of nimodipine (factor A with two levels) and morphine (factor B with five levels) on pain behavior test, as measured by %MPE. The interaction effect between nimodipine and morphine was not statistically significant [$F(4, 90) = 2.37, P > 0.05$]. However, there was a statistically significant effect on pain behavior test for nimodipine [$F(1, 90) = 23.18, P < 0.001$], and morphine [$F(4, 90) = 24.63, P < 0.001$]. Analyses of post-hoc t-test showed that analgesic effect of morphine at doses of 10 and 15 mg/kg plus nimodipine (20 mg/kg) was significantly decreased compared to the respective groups that received morphine plus vehicle (Fig. 3).



NEURSCIENCE

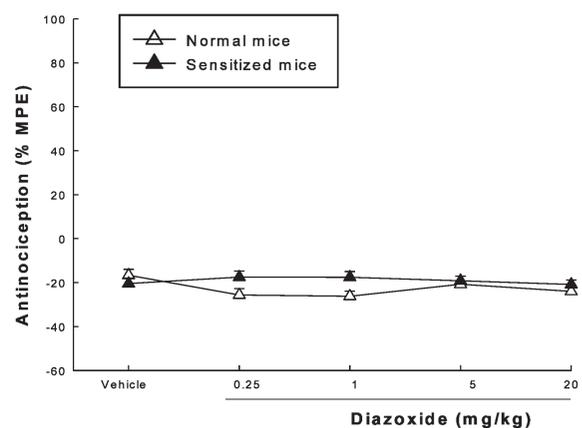
Figure 3. Effects of nimodipine along with morphine on pain behavior test in sensitized mice. Ten groups of animals (n=10) were submitted to the sensitization schedule. On hot-plate test day, five groups of them (points symbolized by white triangle) received vehicle (10 ml/kg) at 45 min before testing plus saline or morphine (5, 7.5, 10 and 15 mg/kg), 30 min before the test. The other five groups (points symbolized by black triangle) in a same way received nimodipine (20 mg/kg) plus saline or morphine (5, 7.5, 10 and 15 mg/kg). Each point is the mean \pm S.E.M. of %MPE. +P<0.05 and \$\$ P<0.01 compared to groups that received vehicle plus the same dose of morphine.

3.4. Diazoxide by itself had no Significant Effect on Pain Behavior Test in either Normal or Sensitized Mice

One-way ANOVA revealed that diazoxide had neither significant analgesic nor algescic effect in both normal mice [one-way ANOVA, $F(4, 45) = 2.58, P > 0.05$], or sensitized mice [one-way ANOVA, $F(4, 45) = 0.4, P > 0.05$] (Fig. 4).

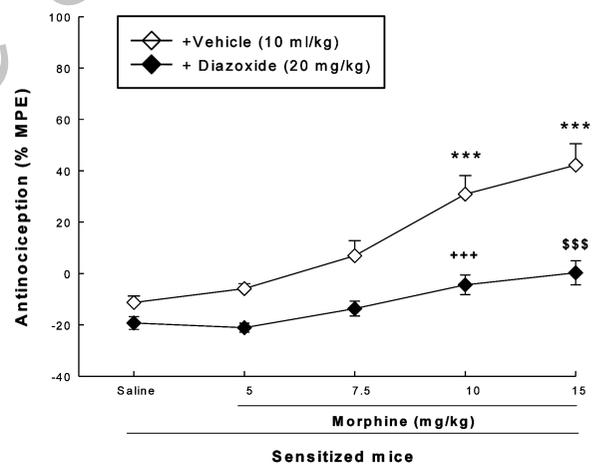
3.5. Diazoxide Caused more Decrease in Analgesic Effect of Morphine in Sensitized Mice

A two-way ANOVA was conducted to explore the effect of diazoxide (factor A with two levels) and morphine (factor B with five levels) on pain behavior test, as measured by %MPE. There was a statistically significant effect for diazoxide [$F(1, 90) = 66.38, P < 0.001$], and for morphine [$F(4, 90) = 23.85, P < 0.001$]. The interaction effect between diazoxide and morphine was also statistically significant [$F(4, 90) = 4.5, P < 0.01$]. Analyses of post-hoc t-test showed that analgesic effect of morphine at doses of 10 and 15 mg/kg was significantly decreased by co-injections of diazoxide (20 mg/kg) compared to the respective groups that received morphine plus vehicle (Fig. 5).



NEURSCIENCE

Figure 4. Effects of diazoxide by itself on pain behavior test in normal and sensitized mice. Ten groups of animals (n=10) were used, and divided into five groups of normal (points symbolized by white square) and five groups of sensitized mice (points symbolized by black triangle). On hotplate test day, each five groups received either vehicle (10 ml/kg) or diazoxide (0.25, 1, 5 and 20 mg/kg), 45 min before examining test latency. Each point is the mean \pm S.E.M. of %MPE.



NEURSCIENCE

Figure 5. Effects of diazoxide along with morphine on pain behavior test in sensitized mice. Five groups of sensitized animals (points symbolized by white triangle) received vehicle (10 ml/kg) plus saline or morphine (5, 7.5, 10 and 15 mg/kg) with 15 min intervals, and test latency was performed for each animal 30 min after the last injections (Note: data from these five groups also used in Fig. 3). The other five sensitized groups (points symbolized by black diamond) in a same way received diazoxide (20 mg/kg) plus saline or morphine (5, 7.5, 10 and 15 mg/kg). Each point is the mean \pm S.E.M. of %MPE. +++P<0.001 and \$\$\$ P<0.001 compared to groups that received vehicle plus the same dose of morphine.

4. Discussion

The results of the present experiments showed that morphine dose-dependently induced analgesia in normal mice, but its analgesic effect was decreased in mice with pretreatment of 3 days morphine followed by 5 days washout. Although the analgesic effect of morphine is well known, however, there are many reports about decreasing morphine-induced analgesia after chronic use of the drug, which is known as opioid-induced hyperalgesia or nociceptive sensitization induced by the opioid (Mao, Price & Mayer, 1995; Silverman, 2009). The effects of morphine have been shown to be mediated primarily through activation of mu-opioid receptors (Bodnar, 2011). In support of this claim, it has been shown that the analgesic, rewarding, and withdrawal-induced aversive effects of morphine are eliminated in mice disrupted for mu-opioid receptors (Matthes et al., 1996; Sora et al., 1997). The present results also showed that analgesic effect of morphine in normal mice was prevented by co-injections of naloxone along with morphine on hotplate test day, which confirm that analgesic effect of morphine is mediated through mu-opioid receptors.

We used a previously reported regimen in mice receiving pretreatment of 3-days morphine (high concentration) followed by 5-days washout to induce nociceptive sensitization (Rezayof, Assadpour & Ali janpour, 2013; Zarrindast & Rezayof, 2004). According to our results, the above used regimen of morphine induced a nociceptive sensitization as revealed by a decrease of analgesic effect of morphine in sensitized mice, one day after the regimen. This result may also be reported as opioid-induced hyperalgesia after a nociceptive sensitization. One possibility for decrease in the analgesic effect of morphine after the nociceptive sensitization in the present study may be due to an increase in pain perception resulted from changes in mu-opioid receptors and related signaling pathways in nociceptive neurons. It has been also reported that opioid-induced hyperalgesia may result from insensitivity to opioids (Garnier et al., 2003). It has been shown that multiple signal transduction pathways including protein kinases, second messengers such as cAMP and ion channels may be engaged once mu-opioid receptors is activated with morphine (Freye & Latasch, 2003; Koch & Hollt, 2008; Liu & Anand, 2001). Therefore, it is possible that not only changes in mu-opioid receptors but also in its downstream signaling molecules including ion channels may underlie the altered analgesic efficacy of morphine.

According to a research, repeated exposure to drugs of abuse such as morphine also results in locomotor sensi-

zation, which leads to an increase in locomotor response (Valjent et al., 2010). Therefore, an alternate possibility for decrease of analgesic effect of morphine after nociceptive sensitization may result from changes in locomotor activity. However, we did not examine locomotor activity, and the above suggested possibility needs more experiments to be clarified. We have previously reported the involvement of L-type calcium channels (Ca²⁺ channels) and ATP-sensitive potassium channels (K⁺ channels) in hyperalgesia induced by morphine in diabetic mice. Therefore, we hypothesize that these channels may also play a role in the hyperalgesia induced by morphine after nociceptive sensitization.

The results of the present study revealed that nimodipine, a blocker of L-type Ca²⁺ channels by itself had neither algesic nor analgesic effect in both normal and sensitized mice. The results also showed that the higher dose of nimodipine along with morphine caused more decrease in analgesic effect of morphine in sensitized mice. Several studies reported that administration of Ca²⁺ channel blockers induce analgesia in laboratory animals (Horvath, Brodacz & Holzer-Petsche, 2001; Miranda et al., 1992). However, in the present study nimodipine by itself did not induce either algesic or analgesic effect in both normal and sensitized mice. Other studies also reported that nimodipine improves analgesic effect of morphine in normal animals (Kumar, Mehra & Ray, 2010; Michaluk, Karolewicz, Antkiewicz-Michaluk & Vetulani, 1998; Shimizu et al., 2004; Zharkovsky, Katajamaki, Seppala & Ahtee, 1999). In the present study, we administered nimodipine along with morphine to investigate possible involvement of Ca²⁺ channels in the decreased analgesic effect of morphine in sensitized mice. An explanation for decreasing the analgesic effects of morphine with nimodipine in sensitized mice may be due to the fact that Ca²⁺ channels are affected by pretreatment regimen of morphine sensitization. However, some other factors such as different subjects, different tests for examining pain behavior and drug doses may account for the discrepant results of this study with others.

The results of the present study also indicated that activation of K⁺ATP channels by diazoxide, an opener of K⁺ATP channel induced neither algesic nor analgesic effects in both normal and sensitized mice. The results also showed that the higher dose of diazoxide along with morphine on hotplate test day caused significant interaction with morphine to decrease the analgesic effect of the later drug in sensitized mice. It has been reported that peripheral analgesic effectiveness of morphine is greatly abrogated during neuropathic pain states (Rashid, Inoue, Matsumoto & Ueda, 2004), and the reduction in K⁺ATP

currents have been reported for the reduced analgesic effectiveness of morphine (Cunha et al., 2010). Since diazoxide caused more decrease in analgesic effect of morphine, we propose that K⁺ATP channels in sensitized mice have been influenced by pretreatment of morphine. It has been reported that intracerebroventricular administrations of the K⁺ATP channel blockers antagonize the analgesic effects of opioids (Ocana, Del Pozo, Barrios & Baeyens, 1995; Ocana, Del Pozo, Barrios, Robles & Baeyens, 1990; Wild, Vanderah, Mosberg & Porreca, 1991). Our results also showed that diazoxide antagonized the analgesic effect of morphine in sensitized mice. Blockade of KATP channels has been proposed to suppress the activation of descending noradrenergic system induced by i.c.v. injections of morphine (Narita et al., 1992). In this study, we examined the effects of K⁺ATP channels and L-type Ca²⁺ channels in morphine-induced hyperalgesia in a diabetic mice model (Ahmadi, Ebrahimi, Oryan & Rafieenia, 2013), and it was observed that the blocker of these channels decreased the diabetes-induced hyperalgesia. On the contrary, the present results showed that nimodipine and diazoxide increased the hyperalgesia in sensitized mice, as revealed by a more decrease in analgesic effect of morphine after a regimen of nociceptive sensitization. Comparing these results with our previous study, it can be proposed that there are different mechanisms underlying the hyperalgesia induced by being either diabetic or sensitized mice models.

5. Conclusions

In summary, the analgesic effect of morphine was decreased in sensitized mice. Nimodipine and diazoxide decreased analgesic effect of higher doses of morphine in sensitized mice. It can be concluded that analgesic effect of morphine after sensitization have been influenced by K⁺ATP channels and Ca²⁺ channels. Our results propose that potassium and calcium channels may be the potential target points for controlling the pain in patients with chronic use of morphine.

References

- Ahmadi, S., Ebrahimi, S. S., Oryan, S., & Rafieenia, F. (2013). Blockades of ATP-sensitive potassium channels and L-type calcium channels improve analgesic effect of morphine in alloxan-induced diabetic mice. *Pathophysiology*, 19, 171-177.
- Asano, T., Dohi, S., & Iida, H. (2000). Antinociceptive action of epidural K⁺(ATP) channel openers via interaction with morphine and an alpha(2)-adrenergic agonist in rats. *Anesth Analg*, 90, 1146-1151.
- Benedek, G., & Szikszay, M. (1984). Potentiation of thermoregulatory and analgesic effects of morphine by calcium antagonists. *Pharmacol Res Commun*, 16, 1009-1018.
- Benyamin, R., Trescot, A. M., Datta, S., Buenaventura, R., Adlaka, R., & Sehgal, N., et al. (2008). Opioid complications and side effects. *Pain Physician*, 11, S105-120.
- Biala, G., & Weglinska, B. (2006). On the mechanism of cross-tolerance between morphine- and nicotine-induced antinociception: involvement of calcium channels. *Prog Neuropsychopharmacol Biol Psychiatry*, 30, 15-21.
- Bodnar, R. J. (2011). Endogenous opiates and behavior: 2010. *Peptides*, 32, 2522-2552.
- Chiou, L. C., & How, C. H. (2001). ATP-sensitive K⁺ channels and cellular actions of morphine in periaqueductal gray slices of neonatal and adult rats. *J Pharmacol Exp Ther*, 298, 493-500.
- Chu, L. F., Angst, M. S., & Clark, D. (2008). Opioid-induced hyperalgesia in humans: molecular mechanisms and clinical considerations. *Clin J Pain*, 24, 479-496.
- Contreras, E., Tamayo, L., & Amigo, M. (1988). Calcium channel antagonists increase morphine-induced analgesia and antagonize morphine tolerance. *Eur J Pharmacol*, 148, 463-466.
- Cunha, T. M., Roman-Campos, D., Lotufo, C. M., Duarte, H. L., Souza, G. R., Verri, W. A., Jr., et al. (2010). Morphine peripheral analgesia depends on activation of the PI3Kgamma/AKT/nNOS/NO/KATP signaling pathway. *Proc Natl Acad Sci U S A*, 107, 4442-4447.
- Del Pozo, E., Caro, G., & Baeyens, J. M. (1987). Analgesic effects of several calcium channel blockers in mice. *Eur J Pharmacol*, 137, 155-160.
- Dogru, A., Yesilyurt, O., Isimer, A., & Guzeldemir, M. E. (2001). L-type and T-type calcium channel blockade potentiate the analgesic effects of morphine and selective mu opioid agonist, but not to selective delta and kappa agonist at the level of the spinal cord in mice. *Pain*, 93, 61-68.
- DuPen, A., Shen, D., & Ersek, M. (2007). Mechanisms of opioid-induced tolerance and hyperalgesia. *Pain Manag Nurs*, 8, 113-121.
- Freye, E., & Latasch, L. (2003). [Development of opioid tolerance -- molecular mechanisms and clinical consequences]. *Anesthesiol Intensivmed Notfallmed Schmerzther*, 38, 14-26.
- Garnier, M., Zaratini, P. F., Ficalora, G., Valente, M., Fontanella, L., & Rhee, M. H., et al. (2003). Up-regulation of regulator of G protein signaling 4 expression in a model of neuropathic pain and insensitivity to morphine. *J Pharmacol Exp Ther*, 304, 1299-1306.

- Horvath, G., Brodacz, B., & Holzer-Petsche, U. (2001). Role of calcium channels in the spinal transmission of nociceptive information from the mesentery. *Pain*, 93, 35-41.
- Kamei, J., Kawashima, N., Narita, M., Suzuki, T., Misawa, M., & Kasuya, Y. (1994). Reduction in ATP-sensitive potassium channel-mediated antinociception in diabetic mice. *Psychopharmacology (Berl)*, 113, 318-321.
- Koch, T., & Hollt, V. (2008). Role of receptor internalization in opioid tolerance and dependence. *Pharmacol Ther*, 117, 199-206.
- Kumar, R., Mehra, R., & Ray, S. B. (2010). L-type calcium channel blockers, morphine and pain: Newer insights. *Indian J Anaesth*, 54, 127-131.
- Lee, M., Silverman, S. M., Hansen, H., Patel, V. B., & Manchikanti, L. (2011). A comprehensive review of opioid-induced hyperalgesia. *Pain Physician*, 14, 145-161.
- Liu, J. G., & Anand, K. J. (2001). Protein kinases modulate the cellular adaptations associated with opioid tolerance and dependence. *Brain Res Brain Res Rev*, 38, 1-19.
- Mao, J., Price, D. D., & Mayer, D. J. (1995). Mechanisms of hyperalgesia and morphine tolerance: a current view of their possible interactions. *Pain*, 62, 259-274.
- Matthes, H. W., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., & Kitchen, L., et al. (1996). Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature*, 383, 819-823.
- Michaluk, J., Karolewicz, B., Antkiewicz-Michaluk, L., & Vetulani, J. (1998). Effects of various Ca²⁺ channel antagonists on morphine analgesia, tolerance and dependence, and on blood pressure in the rat. *Eur J Pharmacol*, 352, 189-197.
- Miranda, H. F., Bustamante, D., Kramer, V., Pelissier, T., Saavedra, H., & Paeile, C., et al. (1992). Antinociceptive effects of Ca²⁺ channel blockers. *Eur J Pharmacol*, 217, 137-141.
- Narita, M., Suzuki, T., Misawa, M., Nagase, H., Nabeshima, A., & Ashizawa, T., et al. (1992). Role of central ATP-sensitive potassium channels in the analgesic effect and spinal noradrenaline turnover-enhancing effect of intracerebroventricularly injected morphine in mice. *Brain Res*, 596, 209-214.
- Nestler, E. J. (2004). Molecular mechanisms of drug addiction. *Neuropharmacology*, 47 Suppl 1, 24-32.
- Ocana, M., Del Pozo, E., Barrios, M., & Baeyens, J. M. (1995). Subgroups among mu-opioid receptor agonists distinguished by ATP-sensitive K⁺ channel-acting drugs. *Br J Pharmacol*, 114, 1296-1302.
- Ocana, M., Del Pozo, E., Barrios, M., Robles, L. I., & Baeyens, J. M. (1990). An ATP-dependent potassium channel blocker antagonizes morphine analgesia. *Eur J Pharmacol*, 186, 377-378.
- Omote, K., Sonoda, H., Kawamata, M., Iwasaki, H., & Namiki, A. (1993). Potentiation of antinociceptive effects of morphine by calcium-channel blockers at the level of the spinal cord. *Anesthesiology*, 79, 746-752.
- Raffa, R. B., & Pergolizzi, J. V. Jr. (2012). Multi-mechanistic analgesia for opioid-induced hyperalgesia. *J Clin Pharm Ther*, 37, 125-127.
- Rashid, M. H., Inoue, M., Matsumoto, M., & Ueda, H. (2004). Switching of bradykinin-mediated nociception following partial sciatic nerve injury in mice. *J Pharmacol Exp Ther*, 308, 1158-1164.
- Rezayof, A., Assadpour, S., & Alijanpour, S. (2013). Morphine-induced anxiolytic-like effect in morphine-sensitized mice: involvement of ventral hippocampal nicotinic acetylcholine receptors. *Pharmacol Biochem Behav*, 103, 460-466.
- Rodrigues, A. R., & Duarte, I. D. (2000). The peripheral antinociceptive effect induced by morphine is associated with ATP-sensitive K⁽⁺⁾ channels. *Br J Pharmacol*, 129, 110-114.
- Shimizu, N., Kishioka, S., Maeda, T., Fukazawa, Y., Yamamoto, C., & Ozaki, M., et al. (2004). Role of pharmacokinetic effects in the potentiation of morphine analgesia by L-type calcium channel blockers in mice. *J Pharmacol Sci*, 94, 240-245.
- Silverman, S. M. (2009). Opioid induced hyperalgesia: clinical implications for the pain practitioner. *Pain Physician*, 12, 679-684.
- Somogyi, A. A., Barratt, D. T., & Collier, J. K. (2007). Pharmacogenetics of opioids. *Clin Pharmacol Ther*, 81, 429-444.
- Sora, I., Takahashi, N., Funada, M., Ujike, H., Revay, R. S., & Donovan, D. M., et al. (1997). Opiate receptor knockout mice define mu receptor roles in endogenous nociceptive responses and morphine-induced analgesia. *Proc Natl Acad Sci U S A*, 94, 1544-1549.
- Sukriti, Hota, D., & Pandhi, P. (2004). Potentiation of antihyperalgesic activity of diclofenac by nimodipine in a formalin model of facial pain in rats. *Methods Find Exp Clin Pharmacol*, 26, 253-256.
- Valjent, E., Bertran-Gonzalez, J., Aubier, B., Greengard, P., Herve, D., & Girault, J. A. (2010). Mechanisms of locomotor sensitization to drugs of abuse in a two-injection protocol. *Neuropsychopharmacology*, 35, 401-415.
- Voitenko, N. V., Kruglikov, I. A., Kostyuk, E. P., & Kostyuk, P. G. (2000). Effect of streptozotocin-induced diabetes on the activity of calcium channels in rat dorsal horn neurons. *Neuroscience*, 95, 519-524.
- Wild, K. D., Vanderah, T., Mosberg, H. I., & Porreca, F. (1991). Opioid delta receptor subtypes are associated with different potassium channels. *Eur J Pharmacol*, 193, 135-136.
- Zarrindast, M. R., & Rezayof, A. (2004). Morphine state-dependent learning: sensitization and interactions with dopamine receptors. *Eur J Pharmacol*, 497, 197-204.
- Zharkovsky, A., Katajamaki, J., Seppala, T., & Ahtee, L. (1999). Morphine-induced analgesia in rats withdrawn from concurrent nimodipine and morphine treatment. *Pain*, 79, 217-222.