Effects of capsazepine, a transient receptor potential vanilloid type 1 antagonist, on morphine-induced antinociception, tolerance, and dependence in mice

T.-L. Nguyen¹, Y.-S. Nam¹, S.-Y. Lee¹, H.-C. Kim² and C.-G. Jang¹*

¹ Department of Pharmacology, School of Pharmacy, Sungkyunkwan University, Suwon 440-746, Republic of Korea
² Neurotoxicology Program, College of Pharmacy, Korea Institute of Drug Abuse, Kangwon National University, Chunchun 200-701, Republic of Korea

* Corresponding author. E-mail: jang@skku.edu

Key points
- Transient receptor potential vanilloid type 1 (TRPV1) receptors may be involved in morphine tolerance.
- In mice, the TRPV1 antagonist capsazepine blocked morphine tolerance and dependence in mice.
- TRPV1 antagonists may be clinically useful.

Background. Repeated morphine treatment has been shown to induce transient receptor potential vanilloid type 1 (TRPV1) expression in the spinal cord, dorsal root ganglion (DRG), and sciatic nerve of a rat model. Increased TRPV1 expression may therefore play a role in morphine tolerance. In this study, we evaluated the hypothesis that blockade of TRPV1 may be useful as an adjunctive pain management therapy. We investigated whether blockage of TRPV1 by capsazepine, a TRPV1 antagonist, affected antinociception, development of tolerance, and physical dependence on morphine in mice.

Methods. Institute of Cancer Research mice were pretreated with capsazepine and post-treated with morphine acutely and repeatedly. Antinociception and its tolerance were assessed using the hot-plate test. Morphine dependence was examined through the manifestation of withdrawal symptoms induced by naloxone in morphine-dependent mice.

Results. Acute capsazepine treatment (5 mg kg⁻¹, i.p.) potentiated the antinociceptive effects of morphine, as measured by the hot-plate test. Repeated co-treatment of capsazepine (2.5 mg kg⁻¹ i.p.) with morphine attenuated the development of tolerance to the antinociceptive effect of morphine. The development of morphine dependence was also reduced by capsazepine (1.25 or 2.5 mg kg⁻¹ i.p.).

Conclusions. Our results suggest that TRPV1 antagonists can be used adjunctively to morphine treatment because they strengthen morphine antinociception and prevent the development of tolerance, and also physical dependence, on morphine.

Keywords: capsazepine; hot-plate test; morphine; transient receptor potential vanilloid type 1; withdrawal symptoms

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Morphine is a potent analgesic used to alleviate moderate or severe pain. However, its use is limited by adverse effects, including analgesic tolerance and physical dependence. Tolerance develops rapidly with repeated use in both laboratory animals and humans and is known to reduce analgesic efficacy. The continuous use of a drug to achieve physiological equilibrium is characterized as physical dependence and is evidenced by withdrawal symptoms after stopping the drug use. The propensity of opioids to trigger marked tolerance and dependence seriously undermines their use in chronic pain management.

The transient receptor potential vanilloid type 1 (TRPV1) receptor is a ligand-gated, non-selective cation channel that is an important integrator of pain stimuli such as endogenous lipids, capsaicin, heat, and low pH. TRPV1 receptors are present in both the peripheral nervous system and the central nervous system (CNS). In the brain, TRPV1 receptors are present in regions that regulate pain transmission and modulation and those that control autonomic functions. Capsazepine is a TRPV1 antagonist that competitively inhibits capsaicin-mediated responses in isolated dorsal root ganglion (DRG) neurones or in tissues from rats and mice. However, early studies that investigated the potential analgesic effects of capsaicine in rat models of acute and chronic pain suggested that capsaicine alone is unlikely to be useful as an analgesic. Thus, TRPV1 antagonists may only be useful in conjunction with other analgesics.

Opioid and TRPV1 receptor agonists have opposing effects: capsaicin treatment blocks the antinociception effects of morphine, μ-opioid receptor agonist, in rats. Capsaicin-induced thermal allodynia is attenuated by stimulating μ-opioid receptors in the CNS and peripheral nervous system of rhesus monkeys.
in vitro binding of peptides selective for μ- and κ-opioids and nociception receptors. These effects can be reversed by capsazepine. TRPV1 and μ-opioid receptors co-localize in DRG neurones and in the spinal cord. The reciprocal interaction between opioid agonists and TRPV1 antagonists suggests that they may act synergistically. In addition, the TRPV1 agonist capsaicin can alter morphine withdrawal symptoms in rats.

Two recent publications reported that blocking or deletion of the TRPV1 receptors could prevent the development of morphine tolerance in rats. However, it is unclear whether blockage of TRPV1 receptors by TRPV1 antagonists can modulate morphine-induced analgesic effects, morphine tolerance, and withdrawal syndromes in mice. Therefore, in the present study, we tested the hypothesis that TRPV1 antagonists may be useful as an adjunctive therapy to enhance morphine analgesic effects and reduce morphine tolerance and dependence in mice. In particular, we investigated whether capsazepine influenced morphine-induced antinociception, development of tolerance, and dependence in mice.

**Methods**

**Animals**

Male Institute of Cancer Research mice (MJ Ltd Co., Seoul, Republic of Korea) weighing 22–28 g were used in all experiments. All animals were acclimatized for 1 week before the experiments and were used only once. Mice were maintained in an animal room under a 12 h light/dark cycle at 23 (± 1) °C. All animal care procedures were conducted in accordance with the US National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals, and our protocol was approved by the Institutional Animal Care and Use Committee of Sungkyunkwan University.

We used a total of 195 mice in this study. For all experiments, mice were randomly divided into groups, and their behaviours were observed by an investigator blinded to the treatments the mice had received. All experiments were carried out between 9:00 a.m. and 1:00 p.m. After experimentation, mice were humanly killed via injection of pentobarbital (100 mg kg⁻¹).

**Drugs**

Capsazepine (Tocris Cookson, Bristol, UK) was dissolved in saline containing 2% dimethyl sulphoxide (DMSO: Sigma Chemical, Poole, Dorset, UK) and 10% Tween 80. Morphine hydrochloride (Macfarlan Smith Ltd, Edinburgh, UK) and naloxone (Sigma Chemical) were also dissolved in physiologically saline.

**Measurement of capsazepine antinociception**

Basal nociceptive response was determined for each mouse on the test day using a hot-plate apparatus in a plastic cylinder (height: 20 cm, diameter: 14 cm). Mice were individually placed onto the hot plate (52 °C), and the time for a mouse to lick a hind paw or jump was recorded (latency). A cut-off time of 40 s was set to prevent tissue damage. Thirty minutes after measuring baseline latency, mice were injected with either vehicle (2% DMSO, 10% Tween 80 in saline) or capsazepine (1.25, 2.5, or 5 mg kg⁻¹, i.p.). Capsazepine doses were based on our preliminary data (data not shown). Mice were then retested after delays of 60, 90, 120, and 150 min.

**Measurement of morphine antinociception**

Morphine analgesia was also measured using the hot-plate test. Thirty minutes after measuring baseline latency, mice were pre-injected with either vehicle or capsazepine (0.625, 1.25, 2.5, or 5 mg kg⁻¹, i.p.). Thirty minutes later, mice were post-injected with saline or morphine (5 mg kg⁻¹, i.p.) and tested after delays of 30, 60, 90, and 120 min. Dosages and time points were chosen based on a preliminary study (data not shown).

**Measurement of the development of morphine tolerance**

In the second part of this study, we examined the effects of repeated capsazepine pretreatment on antinociceptive tolerance to long-term morphine administration. Morphine (10 mg kg⁻¹, s.c.) was administered to mice once a day for 5 days in order to produce tolerance. Thirty minutes before each morphine injection, mice were pretreated with injections of capsazepine (0.625, 1.25, or 2.5 mg kg⁻¹, i.p.) or vehicle. On day 6, the effects of capsazepine on antinociceptive tolerance to morphine (5 mg kg⁻¹, s.c) were evaluated using the hot-plate test.

Antinociceptive response was calculated as a percentage of the maximum possible effect (%MPE): %MPE = [(Tc – To)/ (To – T0)] × 100, where T0 and Tc are the hot-plate paw-licking or jumping latencies before and after morphine injection, respectively. The cut-off time (Tc) was set at 40 s. Effects were measured by calculating the area under the curve (AUC) in a plot of the %MPE (ordinate) vs time (min, abscissa). The AUC was calculated using trapezoidal integration implemented in Microsoft Excel and was expressed as the percentage of the AUC in the control animals.

**Measurement of physical dependence on morphine**

Mice were treated with morphine (10 mg kg⁻¹) once a day at approximately 9:00 a.m. for 7 days to produce dependence. In each case, mice were pretreated with capsazepine (1.25 or 2.5 mg kg⁻¹, i.p.) or vehicle 30 min before morphine injection. On the eighth day, 24 h after the final morphine injection, withdrawal syndromes were induced by injection of an opioid receptor antagonist, naloxone (5 mg kg⁻¹, i.p.). Each animal was immediately placed in a transparent acrylic cylinder (diameter 30 cm) for a 30 min observation of withdrawal manifestations (frequency of jumping and rearing).

**Statistical analyses**

Data are expressed as mean and standard deviation (SD). Data were analysed using one-way analysis of variance (ANOVA) followed by a Newman–Keuls test and two-way
ANOVA followed by Bonferroni’s multiple comparison tests. In the Prism software (Graphpad Software, Inc.) used in this study, treatment was the intersubject factor, and repeated measures with time was the intrasubject factor. A \( P \)-value of <0.05 was considered statistically significant.

Results

Effect of capsazepine on acute morphine antinociception

Capsazepine (1.25, 2.5, or 5 mg kg\(^{-1}\), i.p.) alone had no antinociceptive effect, according to the hot-plate test (Fig. 1). However, analysis of the time courses of antinociception for morphine alone and co-treatment with morphine and capsazepine revealed significant drug and time effects (drug, \( F_{(5188)}=10.25, P<0.0001 \); time, \( F_{(15188)}=65.23, P<0.0001 \); drug \times time interaction, \( F_{(15188)}=3.04, P<0.0001 \); two-way ANOVA; Table 1). Morphine-treated mice showed a significant increase in antinociception (%MPE) compared with that of saline-treated mice for up to 30 min [mean difference 46.0, 95% confidence interval (CI) 9.2–82.7, \( P<0.001 \)], and co-treatment with 5 mg kg\(^{-1}\) capsazepine further increased this antinociception (mean difference 39.7, 95% CI 5.6–73.8, \( P<0.001 \), Table 1).

Acute morphine treatment alone produced significant antinociception (+341%) vs that of the controls (100%) (\( P<0.05 \), Fig. 2). This antinociception was not significantly changed by pretreatment with low doses of capsazepine (0.625, 1.25, or 2.5 mg kg\(^{-1}\)) (Fig. 2). However, capsazepine pretreatment at 5 mg kg\(^{-1}\) significantly increased analgesia (+658%) compared with that of morphine alone (+341%) (\( P<0.01 \), Fig. 2).

Effect of capsazepine on the development of analgesic tolerance to morphine

Analysis of the time courses of antinociception for morphine alone and for co-treatment with morphine and capsazepine revealed significant drug and time effects (drug, \( F_{(5232)}=13.82, P<0.001 \); time, \( F_{(2322)}=5.38, P<0.01 \); drug \times time interaction, \( F_{(15232)}=1.03, P=0.354 \); two-way ANOVA; Table 2). Acute morphine showed significant antinociception at 30 min (mean difference 55.3, 95% CI 20.5–90.0, \( P<0.001 \)) and 60 min (mean difference 33.8, 95% CI 1.0–68.5, \( P<0.05 \)), and co-treatment with 2.5 mg kg\(^{-1}\) capsazepine showed significant antinociception at 60 min (mean difference 31.4, 95% CI –7.2 to 69.96, \( P<0.05 \)). Animals treated with morphine for 5 days (10 mg kg\(^{-1}\)) showed significantly lower analgesic responses at 30 min (mean difference 51.4, 95% CI 25.5–78.6, \( P<0.001 \)), 60 min (mean difference 39.5, 95% CI 10.9–68.0, \( P<0.001 \)), and 90 min (mean difference 24.2, 95% CI –4.3 to 52.8, \( P<0.05 \)) when challenged with 5 mg kg\(^{-1}\) of morphine. However, the antinociceptive responses of mice after chronic administration of capsazepine (2.5 mg kg\(^{-1}\)) with morphine were significantly higher than those of morphine-tolerant mice at 60 min (mean difference 37.1, 95% CI 3.1–71.0, \( P<0.01 \)) and 90 min (mean difference 28.1, 95% CI –5.8 to 62, \( P<0.05 \)) (Table 2).

Morphine tolerance was observed when mice had received repeated morphine treatment for 5 days (+88.70%)

![Fig 1 Effects of capsazepine (CPZ) on nociceptive behaviours in mice. The analgesic effect was determined by calculating the AUC, obtained from a plot of analgesic percentage (ordinate) vs time in minutes (abscissa), and was expressed as a percentage of the effect observed in vehicle-treated control animals (100%). Values indicate the means (so) of seven to eight mice.](image)

<table>
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<th>Treatment</th>
<th>%MPE measured at indicated time points</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
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<tr>
<td>VEH+SAL</td>
<td></td>
<td>7.4 (13.8)</td>
<td>13.4 (20.7)</td>
<td>4.7 (9.2)</td>
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<td>VEH+MORS</td>
<td></td>
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<td>17.3 (15.4)</td>
<td>10.9 (13.6)</td>
<td>6.4 (8.7)</td>
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<td>42.0 (44.0)*</td>
<td>17.1 (26.3)</td>
<td>12.3 (23.9)</td>
<td>11.4 (22.2)</td>
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<td>21.7 (18.9)</td>
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<td>35.3 (39.5)</td>
<td>28.4 (32.0)</td>
<td>13.1 (24.1)</td>
</tr>
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</table>

*\( P<0.05 \), **\( P<0.01 \), and ***\( P<0.001 \) vs the time-matched saline-treated group; **\( P<0.01 \) vs the time-matched saline-treated group; ***\( P<0.001 \) vs the time-matched morphine-treated group (MOR).
compared with those that were acutely treated with morphine (+522.27%) (P<0.001, Fig. 3). However, this antinociceptive tolerance was blocked by co-treatment with capsazepine (2.5 mg kg\(^{-1}\)) for 5 days (P<0.01, Fig. 3).

### Effect of capsazepine on naloxone-precipitated withdrawal syndrome in morphine-dependent mice

Naloxone induced robust jumping and rearing behaviours over the ensuing 30 min after challenge with naloxone (P<0.05, Fig. 4). Repeated pretreatment with capsazepine (1.25 or 2.5 mg kg\(^{-1}\)) 30 min before morphine injection significantly attenuated the jumping (both doses P<0.05, Fig. 4a) and rearing (P<0.05 and <0.01, respectively, Fig. 4a) behaviours.

**Discussion**

We investigated whether the TRPV1 antagonist, capsazepine, altered morphine-induced antinociception, tolerance, and dependence in mice. We found that capsazepine not only enhanced acute analgesia but also suppressed analgesic tolerance and physical dependence on morphine.

Capsazepine blocks hyperalgesia induced by tibial osteosarcoma, capsacin, and carrageen in mice, rats, and guinea pigs. However, capsazepine (up to 100 mg kg\(^{-1}\)) alone does not change pain thresholds in naïve animals, suggesting that it does not have intrinsic analgesic properties. TRPV1 is co-expressed with many other receptors that are also activated by chemokines and cytokines at sensory terminals such as histamine 1 receptors, purine receptors (P2X), acid-sensing ion channels, interleukin 1 receptors, and prostaglandin E\(_2\) receptors. Therefore, selective blockage of TRPV1 receptors by capsazepine may not be sufficient to prevent pain sensation and may explain why capsazepine alone does not increase the pain threshold. This hypothesis is supported by our results (up to 5 mg kg\(^{-1}\) capsazepine) and those of other investigators (up to 100 mg kg\(^{-1}\) capsazepine) that demonstrate that capsazepine does not change the pain thresholds in naïve animals. Furthermore, in our study, acute treatment with capsazepine alone did not induce analgesia, but morphine-induced antinociception was increased in a dose-dependent manner by co-treatment with capsazepine. The TRPV1 antagonist SB366971 strengthens the antinociceptive effects of morphine in a bone cancer pain model in mice.

Our data showed that a TRPV1 antagonist positively interacted with morphine in a thermal pain model. However, the mechanisms underlying the interaction of morphine and capsazepine are unknown. One possibility is that morphine may increase the releases of chemokines and cytokines that counteract its antinociceptive effect. TRPV1 receptors can be modulated by inflammatory mediators, including growth factors, neurotransmitters, peptides or small proteins, lipids, chemokines, and cytokines. Activation of TRPV1 receptors by chemokines and cytokines may cause nociceptive effects that oppose morphine’s...
antinociceptive effects. Therefore, suppressing the nociceptive effects of chemokines and cytokines by capsazepine through blockage of TRPV1 receptors may enhance the effects of morphine.

Chronic administration of morphine results in the development of remarkable tolerance. Although the mechanisms of opioid tolerance are not fully understood, many studies have shown that repeated exposure to morphine increases the releases and expressions of chemokines, pro-inflammatory cytokines, and pronociceptive neurotransmitters in the spinal cord and the DRG, strongly opposing morphine’s analgesic effects. Moreover, chronic morphine treatment increases TRPV1 expressions in the spinal cord, DRG, and sciatic nerve. In bone cancer pain that is resistant to morphine, TRPV1 receptors are up-regulated in DRG neurones. Morphine may induce expression of the TRPV1 receptor through activation of the mitogen-activated protein kinase signalling pathway, which includes upstream regulators of TRPV1. Blockage of TRPV1 receptors by intrathecal administration of SB366971 significantly attenuated morphine tolerance in rats. Similarly, deletion of the TRPV1 receptor-expressing sensory neurones by resiniferatoxin, an ultrapotent capsaicin analogue, blocked morphine tolerance. In agreement with previous studies, our data showed that blockage of TRPV1 receptors with capsazepine (2.5 mg kg⁻¹) inhibited morphine tolerance induced by 5 days of morphine treatment.

Chronic morphine exposure also causes physical dependence that is manifested by withdrawal symptoms. Neuro peptides such as SP and calcitonin CGRP may influence morphine withdrawal, as animals with opioid withdrawal symptoms show high levels of SP and CGRP. Acute intrathecal treatment with SP or CGRP antagonists attenuates morphine withdrawal signs. CGRP-deficient mice show reduced withdrawal-associated jumping, and SP knockout mice have decreased morphine reward and withdrawal. TRPV1 receptors co-localize with SP and CGRP in the primary sensory neurones, spinal cord, and DRG neurones. Capsaicin causes SP and CGRP releases, whereas capsazepine reverses these activities. Therefore, systemic blockade of TRPV1 receptors by capsazepine may diminish withdrawal symptoms by preventing SP and CGRP releases.

To summarize, our data together with those of Niiyama and colleagues indicate that TRPV1 antagonists acutely enhance morphine analgesia. In agreement with previous data on rats, our data on mice also show that TRPV1 antagonists effectively prevent the development of morphine tolerance. Moreover, our data first demonstrated that TRPV1 antagonists significantly reduced withdrawal symptoms in morphine-dependent mice.

In conclusion, our results suggest that a TRPV1 antagonist can be used in combination with morphine to manage chronic and severe morphine-resistant pain and to reduce tolerance and physical dependence on morphine. TRPV1 antagonists can also potentially be used to alleviate morphine withdrawal syndromes.
Conflict of interest
None declared.

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