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 blockage 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

Accepted for publication: 16 June 2010

Morphine is a potent analgesic used to alleviate moderate or severe pain.\(^1\) 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.\(^2\) 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\(^3\) \(^4\) and those that control autonomic functions.\(^5\) Capsazepine is a TRPV1 antagonist that competitively inhibits capsaicin-mediated responses in isolated dorsal root ganglion (DRG) neurones\(^6\) or in tissues from rats\(^7\) and mice.\(^8\) However, early studies that investigated the potential analgesic effects of capsazepine in rat models of acute and chronic pain suggested that capsazepine alone is unlikely to be useful as an analgesic.\(^9\) 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, \(\mu\)-opioid receptor agonist, in rats.\(^10\) Capsaicin-induced thermal allodynia is attenuated by stimulating \(\mu\)-opioid receptors in the CNS and peripheral nervous system of rhesus monkeys.\(^11\) \(^12\) Capsaicin suppresses the
Effects of capsazepine in mice

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 capsacin 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 humanely 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 physiological 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 = [(Tₚ − T₀)/ (Tᵢ − T₀)] × 100, where T₀ and Tᵢ are the hot-plate paw-licking or jumping latencies before and after morphine injection, respectively. The cut-off time (Tᵢ) 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
Fuzepine revealed significant drug and time effects (drug, \( F_{(5,188)} = 10.25, P < 0.001 \); time, \( F_{(3,188)} = 46.53, P < 0.001 \); drug \( \times \) time interaction, \( F_{(15,188)} = 3.04, P < 0.001 \); 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%) \( (P < 0.05, \text{Fig. 2}) \). 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%) \( (P < 0.01, \text{Fig. 2}) \).

**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, \( F_{(5,188)} = 13.82, P < 0.001 \); time, \( F_{(3,188)} = 5.38, P < 0.01 \); drug \( \times \) time interaction, \( F_{(15,188)} = 1.03, P = 0.354 \); 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 69.6, \( P < 0.05 \)). Animals treated with morphine for 5 days (10 mg kg\(^{-1}\)) showed significantly lower analgesic responses at 30 min (mean difference 54.1, 95% CI 25.5–82.6, \( P < 0.001 \)), 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%)}
Effects of capsazepine in mice

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.

![Fig 2 Effects of CPZ on morphine (MOR)-induced analgesia in mice. The analgesic effect was determined by calculating the AUC from a plot of analgesic percentage (ordinate) vs time in minutes (abscissa), and was expressed as a percentage of the effect observed in VEH+SAL-treated control animals (100%). Values indicate the means (so) of eight to 12 mice. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ vs animals treated with VEH+SAL; &$P<0.05$ vs animals treated with VEH+MOR5.](image-url)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%MPE measured at indicated time points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>VEH+SAL+SAL</td>
<td>9.1 (25.8)</td>
</tr>
<tr>
<td>VEH+SAL+MOR5</td>
<td>64.4 (25.0)$^{bc}$</td>
</tr>
<tr>
<td>VEH+MOR10+MOR5</td>
<td>10.3 (12.5)$^{***}$</td>
</tr>
<tr>
<td>CPZ 0.625+MOR10+MOR5</td>
<td>26.7 (30.5)</td>
</tr>
<tr>
<td>CPZ 2.5+MOR10+MOR5</td>
<td>28.8 (34.0)</td>
</tr>
<tr>
<td>CPZ 5+MOR10+MOR5</td>
<td>27.9 (24.1)</td>
</tr>
</tbody>
</table>

Table 2 Effects of CPZ treatment on the development of analgesic tolerance to morphine. CPZ (0.625, 1.25, or 2.5 mg kg$^{-1}$, i.p.) or vehicle (VEH) was used for pretreatment 30 min before treatment with MOR10 (morphine 10 mg kg$^{-1}$, s.c) or saline (SAL) control daily for 5 days. Twenty-four hours after the final injection, mice were challenged with MOR5 (morphine 5 mg kg$^{-1}$, s.c.). Scores are expressed as %MPE. Values indicated means (so) for eight to 15 mice. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ vs the time-matched animals treated with VEH+SAL+MOR5. $^{bc}$,$P<0.05$, $^{ab}$,$P<0.001$ vs the time-matched saline-treated controls. $^P<0.05$, $^{ab}$,$P<0.01$ vs the time-matched animals treated with VEH+MOR10+MOR5.

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, capsaicin, and carrageen in mice, rats, and guinea pigs. However, capsazepine (up to 100 mg kg$^{-1}$) alone does not change pain thresholds in naive 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 (P$_2$X), 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 naive 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 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.

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.

Funding

This study was supported by a KRF grant (KRF-2007-313-E00399) and a KOSEF grant (2008-0060176), and also a grant from the BRC of the 21st Century Frontier Research Program (2010K000812), Republic of Korea.

References

4 Roberts JC, Davis JB, Benham CD. [3H]Resiniferatoxin autoradiography in the CNS of wild-type and TRPV1 null mice defines TRPV1 (VR-1) protein distribution. Brain Res 2004; 995: 176–83
14 Chen SR, Pan HL. Loss of TRPV1-expressing sensory neurons reduces spinal mu opioid receptors but paradoxically potentiates opioid analgesia. J Neurophsyiol 2006; 95: 3086–96

Effects of capsazepine in mice

24 Ma W, Quirion R. Inflammatory mediators modulating the transient receptor potential vanilloid 1 receptor: therapeutic targets to treat inflammatory and neuropathic pain. Expert Opin Ther Targets 2007; 11: 307–20
36 Salmon AM, Damaj MI, Marubio LM, Epping-Jordan MP, Merlo-Pich E, Changeux JP. Altered neuroadaptation in opiate...
37 Murtra P, Sheasby AM, Hunt SP, De Felipe C. Rewarding effects of opiates are absent in mice lacking the receptor for substance P. Nature 2000; 405: 180–3
40 Tang HB, Nakata Y. The activation of transient receptor potential vanilloid receptor subtype 1 by capsaicin without extracellular Ca^{2+} is involved in the mechanism of distinct substance P release in cultured rat dorsal root ganglion neurons. Naunyn Schmiedebergs Arch Pharmacol 2008; 377: 325–32