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

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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

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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, μ-opioid receptor agonist, in rats.[10] Capsaicin-induced thermal allodynia is attenuated by stimulating μ-opioid receptors in the CNS and peripheral nervous system of rhesus monkeys.[11 12] Capsaicin suppresses the

in vitro binding of peptides selective for μ- and κ-opioids and nociception receptors. These effects can be reversed by capsazepine.[13] TRPV1 and μ-opioid receptors co-localize in DRG neurones[14 15] and in the spinal cord.[14] 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.[16–18]

Two recent publications reported that blocking or deletion of the TRPV1 receptors could prevent the development of morphine tolerance in rats.[14 15] 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 (SD ±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 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): $\%MPE = [(T_t - T_o)/(T_c - T_o)] \times 100$, where T_o and T_t are the hot-plate paw-licking or jumping latencies before and after morphine injection, respectively. The cut-off time (T_c) 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