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TRPV1 Receptor in Expression of Opioid-induced hyperalgesia

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Abstract

Opiates are currently the mainstay for treatment of moderate to severe pain. However, prolonged administration of opiates has been reported to elicit hyperalgesia in animals and examples of opiate-induced hyperalgesia have been reported in humans as well. In spite of the potential clinical significance of such opiate-induced actions, the mechanisms of opiate-induced hypersensitivity remain unknown. The TRPV1 receptor, a molecular sensor of noxious heat, acts as an integrator of multiple forms of noxious stimuli and plays an important role in the development of inflammation-induced hyperalgesia. As animals treated with opiates show thermal hyperalgesia, we examined the possible role of TRPV1 receptors in the development of morphine-induced hyperalgesia using TRPV1 wild-type (WT) and knock-out (KO) mice and with administration of a TRPV1 antagonist in mice and rats. Administration of morphine by subcutaneous implantation of morphine pellets elicited both thermal and tactile hypersensitivity in TRPV1 WT mice, but not in TRPV1 KO mice. Moreover, oral administration of a TRPV1 antagonist reversed both thermal and tactile hypersensitivity induced by sustained morphine administration in mice and rats. Immunohistochemical analyses indicate that sustained morphine administration modestly increases TRPV1 labeling in the dorsal root ganglia (DRG). In addition, sustained morphine increased flinching and plasma extravasation after peripheral stimulation with capsaicin, suggesting an increase in TRPV1 receptor function in the periphery in morphine treated animals. Collectively our data indicate that the TRPV1 receptor is an essential peripheral mechanism in expression of morphine-induced hyperalgesia.

PERSPECTIVE—Opioid-induced hyperalgesia possibly limits the usefulness of opioids, emphasizing the value of alternative methods of pain control. We demonstrate that TRPV1 channels play an important role in peripheral mechanisms of opioid-induced hyperalgesia. Such information may lead to the discovery of analgesics lacking such adaptations and improving treatment of chronic pain.

Introduction

Opiate analgesics are the mainstay of pain management in conditions ranging from acute to chronic pain. Clinical uses of opiates, such as treating cancer pain, often require opiate treatment for extended periods of time⁴⁹. A potential problem which has been noted with sustained opiate administration is the paradoxical expression of “pain”. Patients treated with prolonged or high doses of opiates have reported abnormal pain in regions unaffected by the initial pain complaint^{3,15,16}. Clinical studies have reported that opioids administered through different routes of administration (transdermal, oral, i.th., i.v.) can unexpectedly

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produce hyperalgesia and allodynia, particularly during rapid opioid dose escalation^{15,31,48,61,62}, a phenomenon described as an Emerging Iatrogenic Syndrome⁴⁸. Such opioid-induced hyperalgesia may require supplemental opioids to maintain constant levels of antinociception. Despite the potential clinical significance of such opiate-induced adaptations in the nervous system, the mechanisms underlying opioid-induced pain are not well understood^{53,68}.

Many preclinical studies have also demonstrated opioid-induced hyperalgesia^{7,8,35,37,44-46,67,75}. Studies have shown that sustained opiate administration results in numerous pronociceptive changes, including increased content and capsaicin-evoked release of pronociceptive neurotransmitters within the spinal dorsal horn^{22,53,69}. A prominent feature of opioid-induced hyperalgesia is enhanced responsiveness to noxious thermal stimulation suggesting TRPV1 channels may be important in this response.

The TRPV1 receptor belongs to the large family of transient receptor potential (TRP) channels that comprise a diverse group of ligand-gated, non-selective cation channels^{4,66}. It is a molecular transducer of noxious thermal and chemical stimuli such as vanilloids (capsaicin) and acids^{4,6}. Additionally, it is well established that TRPV1 expression plays an important role in the development of inflammation-induced hyperalgesia^{4,5,14,32}. Inflammation and morphine-induced hypersensitivity share many common characteristics such as hyperalgesia, allodynia as well as similar pronociceptive neuroadaptive changes. Increased expression of SP and CGRP in the sensory primary afferents, accompanied by increased capsaicin-evoked release of SP and CGRP in the spinal dorsal horn have been described in both inflammation and morphine-induced hyperalgesia^{1,2,18,19,22,34,35,39,41-43,52,56}. Recently it was demonstrated that inflammation increases TRPV1 expression in the DRG, which is then transported to the peripheral but not central terminals³². Based on the critical role of TRPV1 receptor in the inflammatory pain and similarities between inflammatory and morphine-induced pain, we examined the role of the TRPV1 receptor in the development of sustained morphine-induced hypersensitivity. Our findings indicate that the TRPV1 receptor is an essential peripheral mechanism in expression of morphine-induced hyperalgesia.

Materials and Methods

Animals

Male TRPV1 receptor knock-out (KO) mice (Jackson Laboratory, Bar Harbor, Maine), their wild-type (WT) littermates C57BL6 (Jackson Laboratory, Bar Harbor, Maine), ICR mice (Jackson Laboratory, Bar Harbor, Maine) weighing between 20 and 30 g, and Male Sprague–Dawley rats (Harlan; Indianapolis, IN), 200–300 g were maintained on a 12/12h light/dark cycle and were provided food and water ad libitum. All testing was performed in accordance with the policies and recommendations of the International Association for the Study of Pain and the National Institutes of Health guidelines for the handling and use of laboratory animals and received approval from the Institutional Animal Care and Use Committee of the University of Arizona. Groups of 6-8 mice or rats were used in all experiments.

Sustained morphine administration

Sustained morphine administration was accomplished by subcutaneous implantation of one (in mice) or two (in rats) 75 mg free base morphine pellets on the back 1 inch above the pelvic bone. Control groups received placebo pellets containing excipient only. The pellets were obtained as a generous gift from the National Institute on Drug Abuse Drug Supply Program. The doses of morphine in rats (two pellets) and mice (one pellet) were carried out

according to standard methods^{22,25,57,59,63,64,69,76}. Previous studies have demonstrated that implantation of two morphine pellets (75 mg, free base) in rats results in a steady state of plasma morphine levels by 3 days post pellet implantation, ranging between 86.8 ± 13.1 and 156 ± 45 ng/ml, that was maintained through 12 days post-pellet implant^{25,76}. Mice were visually evaluated for signs of morphine- induced agonist actions by assessing the presence or absence of the Straub tail response and by observing characteristic stereotypic locomotor actions in the home cage^{27,51}. Thermal antinociception was verified 6 h following pellet implantation using Hargreaves test in rats and the hot plate test in mice, since the cage-circling behaviors in mice at these time-points prevented the use of the Hargreaves test in mice. All the experiments with mice were performed on day 7 and with rats on day 6 after pellet implantation. As cage circling behavior in mice was no longer observed 7 days post-pellet implantation, thermal antinociceptive testing was done using Hargreaves test in both rats and mice. No signs of withdrawal (diarrhea, wet-dog shaking, jumping, lacrimation, or piloerection) were observed throughout the study in spite of careful monitoring.

Oral injections

AMG0347, a TRPV1 antagonist, generously provided by Dr. Narender Gavva (AMGEN Inc, Thousand Oaks, CA), was injected 3mg/kg (p.o.); this dose was chosen following pilot experimentation. AMG 0347 was dissolved in 5% ethanol and administered as 0.5% methylcellulose suspension; control animals received the vehicle alone. Animals were tested 120, 150 and 180 min after the injection.

Behavioral analysis

Thermal antinociception and hypersensitivity—Paw withdrawal latencies were measured on a Plantar Test Apparatus (Stoelting) calibrated according to manufacturer specifications to ensure consistent delivery of the noxious thermal stimulus throughout the study. The method of Hargreaves²⁶ was used to assess paw-withdrawal latency to a thermal nociceptive stimulus. Rats or mice were allowed to acclimate within a Plexiglas enclosure on a clear glass plate in a quiet testing room for 30–45 min. A radiant heat source was focused onto the plantar surface of the hind paw. Paw-withdrawal latency was determined by a motion detector that halted both the lamp and timer when the paw was withdrawn. A maximal cut-off time of 32 s was used to prevent tissue damage as previously reported^{21,23,35,67}.

The Hot Plate Analgesia Meter was used to test thermal antinociception in mice 6 hours after implantation of pellets as characteristic morphine-induced hyperlocomotion prevented accurate testing using the Hargreaves testing procedure at this time-point. Mice were allowed to acclimate within the home cage in the testing room for 30–45 min. Mice were placed on the hot plate (52°C), which was surrounded by a clear acrylic cage, and the Start/Stop button on the timer was activated. The latency to respond with either a paw lick, paw flick, or jump (whichever came first) was measured to the nearest 0.1 seconds by deactivating the timer when the response is observed. The mouse was immediately removed from the hot plate and returned to its home cage. A maximal cut-off latency of 32 s was used to prevent tissue damage.

Tactile hypersensitivity—Paw-withdrawal thresholds of the hindpaw were determined in response to probing with calibrated von Frey filaments (Stoelting, Wood Dale, IL) in logarithmically spaced increments ranging from 0.4 to 4.0g for mice and 2–15g for rats. Animals were allowed to acclimate in suspended wire-mesh cages for 30–45 min. Each filament was applied perpendicularly to the plantar surface of the paw. Withdrawal threshold was determined by sequentially increasing and decreasing the stimulus strength (“up and down” method), analyzed using a Dixon nonparametric test^{10,17}, and expressed as the mean

withdrawal threshold. A significant reduction in paw-withdrawal threshold from the pre-pellet value indicated tactile hypersensitivity.

Capsaicin-induced flinching—Rats treated with either placebo or morphine pellets for 6 days, were given an injection (s.c.) of capsaicin (Sigma) (100 μ g/100 μ l), dissolved in 100% ethanol into the plantar surface of the hindpaw. The number of flinches was counted across 5 min post injection. Administration of 100% ethanol alone failed to elicit flinching behavior in placebo or morphine treated rats.

Capsaicin-induced plasma extravasation—Rats treated with placebo or morphine pellets for 6 days, were given an injection (s.c.) (50 μ g/50 μ l) of capsaicin (Sigma), dissolved in 100% ethanol, into the dorsal surface of the hindpaw followed by an injection (100 mg/kg, i.v.) of Evans Blue (Sigma) 20 min later. Two (2) hours later, rats were euthanized, and skin punches (3 mm diameter) were taken and placed overnight in formamide. Samples were read with a spectrophotometer (Beckman Instruments Inc., Fullerton, CA) (λ =620 nm) and data converted to μ g/ml Evans Blue using a standard.

Immunohistochemistry

Immunohistochemistry was performed as previously detailed^{22,35,72}. Briefly, on day 6 following morphine or placebo pellet implant, rats received an overdose of sodium pentobarbital (100 mg/kg, i.p.) and were perfused transcardially with 0.1 M PBS until the exudate ran clear followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Lumbar spinal cords (L4-6), DRG (L-5,6) and sciatic nerve were harvested and postfixed in 4% paraformaldehyde overnight and then cryoprotected with 20% sucrose in 0.1 M PBS. Immunostaining was performed on 20 μ m sections from tissues of rats that had received no nociceptive stimulation prior to tissue collection. DRG and spinal sections were incubated overnight at 4°C with primary antibody for VR1 (Neuromics) diluted 1:10,000. Sections were then incubated in secondary antibody Alexa Fluor 568 goat anti-rabbit IgG (Invitrogen) diluted 1:1000 for 2 hours at room temperature. Every fifth section was picked from a series of consecutive DRG sections (20 μ m) providing 100 μ m between sections to prevent significant overlap of neuronal profiles and minimize repeated counting of the same neuronal profile across multiple sections. Control (placebo) and treated (morphine) DRG sections were processed under the same conditions. The specificity of the TRPV1 antibody was tested by preincubating the primary antibody with the immunizing peptide (Neuromics). Preabsorption completely abolished the IR immunolabeling in the DRGs (data not shown). The binding specificity of the secondary antibody was tested by omitting the primary antibody. None of the sections from this control experiment showed any labeling (data not shown).

Image analysis and quantification

Fluorescence images of spinal cord and DRG sections were acquired with a Nikon E800 fluorescence microscope outfitted with a filter set for Cy3 (excitation 540–580 nm/emission 560–620 nm) and a Hamamatsu C5810 color CCD camera and its proprietary Image Processor software (Hamamatsu Photonic System, Bridgewater, NJ, USA). Digital images were output using Adobe Photoshop 6.0 (Adobe System Inc., San Jose, CA, USA). Immunoreactive neuronal profiles were counted in a blinded fashion on 8-10 randomly selected L5-6 DRG sections from 3 animals per each condition. The results are expressed as a percentage of the estimated total number of neuronal profiles from these sections.

Qualitative analysis of spinal cord sections was performed by comparing 6 sections per spinal cord collected from 3 rats per treatment (morphine vs. placebo).

Data analysis

Pair-wise comparisons were made using Student's t-test. In the case of multiple comparisons, such as time-course analysis, means were compared to baseline values by analysis of variance (ANOVA), followed by post hoc Fisher's Least Significant Difference test for multiple comparisons. A probability level of 0.05 indicates significance for all tests.

Results

TRPV1 KO mice do not develop thermal and tactile hyperalgesia

All mice that received morphine pellets demonstrated Straub tail behavior and showed characteristic cage-circling behavior within 30 min of morphine pellet implantation which lasted several hours post pellet implantation^{27,51}. Both TRPV1 KO and WT mice that received morphine pellets developed thermal antinociception on the hot-plate test 6 hours post implantation (Fig 1A).

Sustained morphine administration produced thermal hypersensitivity, as measured by the Hargreaves paw withdrawal test (Fig 1B), as well as tactile hypersensitivity (Fig. 1C) by 7 days post pellet administration in wildtype mice treated with morphine pellets. In contrast, TRPV1 receptor knock-out mice failed to show thermal or tactile hypersensitivity (Fig. 1B, C). Paw-withdrawal responses in placebo treated mice were not different from baseline values throughout the time course, indicating that the thermal and tactile hypersensitivity observed in morphine treated animals was not due to repeated testing.

TRPV1 antagonist AMG 0347 blocks morphine-induced thermal and tactile hypersensitivity in mice

In order to determine whether blocking the TRPV1 receptor reversed sustained morphine-induced thermal and tactile hypersensitivity in ICR mice, baseline paw withdrawal latencies (sec) and paw withdrawal thresholds (g) were determined in separate groups of animals prior to implantation of morphine and placebo pellets. Baseline paw withdrawal latencies to thermal stimulation ranged from 10.3 ± 0.7 to 11.7 ± 1.0 seconds, with no significant difference between treatment groups. Baseline paw withdrawal thresholds to von Frey filaments ranged from $2.6 \pm .02$ to 2.8 ± 0.1 g, with no significant differences between treatment groups. All mice that received morphine pellets demonstrated Straub tail behavior and showed characteristic cage-circling behavior within 30 min of morphine pellets implantation^{27,51}.

Sustained morphine administration reliably produced thermal hypersensitivity, as measured by the Hargreaves test and tactile hyperalgesia by the 7th day after implantation in ICR mice (Fig. 2 A, B). Oral administration of AMG 0347 (3mg/kg) on day 7 after pellet implantation reversed both thermal and tactile hypersensitivity in ICR mice, with the peak effect observed at 2 hr time point after administration of the TRPV1 antagonist (Fig. 2 A, B). The effect of the TRPV1 antagonist dissipated by the 3 hr time point, with re-established hyperalgesia in the morphine treated animals. Of note, this dose of the TRPV1 receptor antagonist did not induce antinociception in placebo treated mice (Fig. 2 A).

TRPV1 antagonist AMG 0347 blocks morphine induced thermal and tactile hypersensitivity in rats

The ability of the TRPV1 receptor antagonist to reverse sustained morphine-induced thermal and tactile hypersensitivity was also determined in SD rats. Baseline paw withdrawal latencies (sec) and paw withdrawal thresholds (g) were determined in separate groups of animals prior to implantation of morphine and placebo pellets. Baseline paw withdrawal

latencies to thermal stimulation ranged from 19.2 ± 0.3 to 20.7 ± 0.3 seconds, with no significant difference between treatment groups. No mice showed baseline paw withdrawal thresholds to von Frey filaments below the 15 g threshold. All rats that were treated with morphine pellets developed thermal antinociception by 6 hours post-pellet implantation. Animals were tested again on day 6 after pellet implantation. Rats treated with morphine pellets (2×75 mg) developed thermal and tactile hyperalgesia by the 6th day after implantation (Fig 3 A, B). Oral administration of AMG 0347 (3 mg/kg) on day 6 after pellet implantation reversed both thermal and tactile hypersensitivity in rats, with peak effectiveness observed two hrs after the injection (Fig 3 A, B). The effect of the TRPV1 antagonist dissipated within 3 hrs after the injection. As with the mice, this dose of AMG 0347 did not induce analgesia in placebo treated rats (Fig. 3A).

Sustained morphine increases TRPV1 immunoreactivity in DRG neurons but not in the spinal cord

Immunohistochemical staining was performed on tissue collected 6 days after morphine or placebo pellet implantation. Within the DRG, sustained morphine across 6 days, but not 2 days, increased immunofluorescent staining for TRPV1 positive profiles (Fig. 4A). Counts verified a small, but significant (13%), increase in the percentage of TRPV1-ir positive neuronal profiles within the DRG (Fig. 4B).

Immunofluorescent labeling of the TRPV1 receptor in the spinal cord was also studied. Immunofluorescent labeling was unaltered in the spinal dorsal horn of morphine treated animals compared to saline controls 6 days after pellet implantation (Fig. 4C).

Sustained morphine increased capsaicin-induced flinching and plasma extravasation

Functional changes in TRPV1 receptor function were determined by examining the effects of peripheral administration of capsaicin, on pain behavior (flinching) and on plasma extravasation. Sustained morphine across 6 days doubled the number of capsaicin-induced flinches compared to placebo controls (Fig. 5 A). Injection of vehicle (100% ethanol) failed to produce flinching behavior in rats treated with either placebo or morphine pellets (data not shown).

Separate groups of rats received an injection of capsaicin into the dorsal surface of the hindpaw and a tail-vein injection of Evans Blue to determine capsaicin-induced plasma extravasation. Rats receiving morphine treatment showed an approximate 5-fold increase in plasma extravasation compared to placebo treated rats (Fig. 5 B) indicating that sustained morphine administration enhances neurogenic inflammation in response to capsaicin. Very little plasma extravasation was observed in the contralateral hindpaw in both groups, with $1.02 \pm .21$ μ g/ml in the placebo treated rats and $1.34 \pm .21$ μ g/ml in the morphine treated animals. These values were not significantly different from each other. These data suggest that sustained morphine alters function of peripheral TRPV1 receptors.

Discussion

Our findings show that: a) unlike wild-type mice, TRPV1 KO mice do not develop thermal and tactile hypersensitivity induced by sustained morphine administration; b) a TRPV1 antagonist reverses morphine-induced thermal and tactile hypersensitivity in mice and rats; c) sustained morphine increases TRPV1 immunoreactivity in the DRG but not in the spinal cord; and d) sustained morphine induces functional changes in TRPV1 receptor at the periphery, which is determined by an increase in capsaicin-induced flinching and plasma extravasation. These data suggest that the TRPV1 receptor is an essential part of the

peripheral neuroadaptive changes that lead to enhanced pain states (i.e., hyperalgesia) after sustained morphine.

Prolonged opioid therapy may lead to the development of unexpected, abnormal pain in both clinical^{13,15,16,20,61} and preclinical^{8,22,37,45,67,68,74,75} settings. The precise mechanism of the development of morphine-induced hyperalgesia after extended exposure still remains unknown. Recent studies have demonstrated that such pain may be secondary to neuroplastic changes that occur in the brain and spinal cord^{22,67,69}. The data presented here suggest that sustained opioid exposure also enhances TRPV1 receptor function in the periphery which plays an additional and essential role in sustained morphine induced thermal as well as tactile hypersensitivity.

Subcutaneous implantation of morphine pellets produced expected antinociception within the first 2-6 hours in all strains of mice, accompanied by increased locomotion and Straub tail, suggesting that the antinociceptive actions of morphine were not attenuated in the TRPV1 KO mice. The baseline paw withdrawal latencies of TRPV1 KO mice were higher than those of WT mice which is consistent with previously published data⁵. Thermal hyperalgesia and tactile allodynia were readily demonstrated in WT mice receiving continuous morphine treatment, but not TRPV1 KO mice. Our findings indicate that TRPV1 KO mice do not develop morphine-induced tactile and thermal hypersensitivity, suggesting that TRPV1 receptor is essential in the development of hyperalgesia states after sustained administration of morphine.

The data from our experiments with knock-out animals was also confirmed with a specific TRPV1 antagonist, AMG 0347³⁰. AMG 0347 administration blocked the thermal and tactile hyperalgesia developed following sustained morphine administration in ICR mice and SD rats. Several studies have shown the reversal of thermal, tactile and mechanical hypersensitivity after inflammation and nerve injury by a variety of TRPV1 receptor antagonists^{23,55,28,71}, and thus suggest a role of TRPV1 for mediating responses to mechanical stimuli. Moreover, it has long been known that intradermal or topical application of capsaicin induces not only thermal but also mechanical hyperalgesia in a number of species including rodents and humans^{24,36,60,71}. The potential for TRPV1-expressing neurons to be at least indirectly involved in the transmission of mechanical sensory information has been suggested⁴⁰.

Critically, no signs of withdrawal were observed throughout the study in spite of careful monitoring. Previous studies have shown that implantation of two morphine pellets (75 mg, free base) in rats results in a steady state of plasma morphine levels and that such levels were maintained across 12 days^{25,76}. These findings indicate that the observed thermal and tactile hypersensitivity was not a result of withdrawal from morphine. Rather, the data support the importance of TRPV1 in neuroadaptive changes that can result in enhancement of nociceptive input after sustained morphine exposure.

The changes induced by sustained morphine exposure are qualitatively similar to those observed in states of inflammatory injury suggesting that sustained morphine-induced hyperalgesia and inflammatory hyperalgesia are likely to share some common underlying mechanisms³⁵. Both inflammatory pain and sustained morphine-induced hyperalgesia are characterized by up-regulation of spinal dynorphin^{50,58,68}, CGRP and SP in the DRG and spinal cord^{18,19,22,39}, and the NK-1 receptor within the spinal cord^{1,2,35}. It is well established that the TRPV1 receptor plays an important role in the development of inflammatory hyperalgesia^{4,14,32}. TRPV1 KO animals lack the ability to develop carrageenan-induced thermal hyperalgesia¹⁴. Previous studies have shown an increase in TRPV1 expression in the DRGs after peripheral inflammation, which is then transported

peripherally, but not centrally³². Here, we show a small, but significant, increase in the number of TRPV1 receptor positive cells in the DRG, but no apparent change in the spinal cord after sustained treatment with morphine. Together with enhanced functional responses to activation of the TRPV1 receptor in morphine treated rats, these findings would be consistent with the possibility of increased trafficking of the TRPV1 channel to peripheral, but not central terminals. Recent work by Suzuki et al. also supports the peripheral role of TRPV1 receptors in morphine-induced hyperalgesia⁶⁵. Morphine infusion across 7-10 days was shown to dramatically enhance the responsiveness of spinal neurons to thermal stimulation indicated by expanded receptive fields and augmented nociceptive inputs. The hyperexcitability of deep dorsal horn neurons may be due in part to enhanced nociceptive input due to increased responses of peripheral nociceptors due, in part, to increased activity of the TRPV1 channel. In addition, this heightened excitability may reflect compensatory mechanisms in the central nervous system including influences from brainstem excitatory pathways⁶⁸.

While the hypothesis of changes in trafficking is plausible and remains to be explored, as noted above, enhancement of capsaicin-induced flinching behavior as well as increased levels of plasma extravasation demonstrates increased function of TRPV1 receptors after sustained morphine treatment. Potentiation of TRPV1 channel activity nociceptor sensitization and activation that leads to hyperalgesia and enhanced neurogenic inflammation⁵⁴. Our data indicate that TRPV1 receptor also plays a critical role in the development of morphine-induced thermal and tactile hyperalgesia and suggests that sustained morphine exposure may directly or indirectly result in sensitization of primary afferent fibers. Sensitization of nociceptors through a TRPV1 mechanism could occur because the gating of the channel is enhanced due to increased phosphorylation. Studies of inflammation-induced hyperalgesia suggest that nociceptor sensitization may result from both by up-regulation of TRPV1 channels as well as enhanced phosphorylation by p38 MAPK, PKC, PKC γ and Src family kinases^{9,32,33,70,77}. Changes in the phosphorylation state of the TRPV1 channel following prolonged morphine treatment is possible and awaits experimental confirmation.

In addition to the contribution of peripheral mechanisms described above, it should be noted that central adaptations have been observed following opiate administration and that these changes also strongly contribute to the expression of opiate-induced hyperalgesia. A non-inclusive list of pronociceptive substances reported to mediate opioid-induced hyperalgesia includes: substance P (SP) and calcitonin gene-related peptide (CGRP)^{22,35,39,47,56}, glutamate^{8,37,38,45,46}, as well as spinal dynorphin and cholecystokinin (CCK) expression in the rostral ventromedial medulla^{21,22,67-69,73}. The descending pain modulatory pathway from the brainstem rostral ventromedial medulla (RVM) via the dorsolateral funiculus (DLF) has been shown to also be critical for maintaining the changes observed in the spinal cord as well as abnormal pain states after continuous treatment with opioids. Animals with lesion of DLF do not develop abnormal pain upon sustained exposure to opioids⁶⁹. Additionally, it has been shown that sustained morphine-induced plasticity in the RVM activates descending facilitation, in part through enhanced activity of cholecystokinin (CCK) in this region^{68,69,73}. Changes in levels of activation of TRPV1 expressing afferent fibers may be critical in an ascending pathway that ultimately results in the activation of descending pain modulatory systems. Such ascending and descending pathways may form part of a loop which ultimately underlies hypersensitivity in cases of opiate-induced hyperalgesia or after injuries²⁹.

The present data demonstrate the essential role of TRPV1 receptor in the development of hyperalgesic states in rodents after sustained morphine treatment. Current pain management techniques for the management of moderate to severe pain, of both malignant and

nonmalignant origin, rely on opiate administration. Recent studies have shown that deletion of TRPV1 expressing afferent neurons paradoxically potentiates the analgesic efficacy of mu opioid agonists^{11,12}. Hence, the recognition of the contribution of the TRPV1 receptor in opioid-induced hyperalgesia may help to identify new strategies for drug development and combination therapy for opiate use in management of chronic pain.

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Fig. 1.

A) Both TRPV1 KO and WT mice develop morphine induced thermal antinociception, measured as response latency on a 52°C hot plate, within 6 hours of pellet implantation (see Methods). B) The development of morphine induced thermal hypersensitivity was measured using the Hargreaves paw flick test. Antinociception observed at 6 hours was not observed in WT or KO mice 2 days following morphine pellet implantation. WT, but not TRPV1 KO mice developed thermal hypersensitivity by 6 days post-pellet implant. C) Tactile hypersensitivity developed in WT, but not TRPV1 KO mice within 6 days post-pellet implantation. Paw-withdrawal responses in placebo treated animals were not different from baseline values throughout the time course. * indicates significant difference from baseline ($p < 0.05$).

Fig. 2.

A) ICR mice developed morphine-induced thermal hypersensitivity, measured using the Hargreaves paw-flick test, within 7 days of pellet implantation (Pre-AMG). Oral administration of AMG 0347 (3 mg/kg) on day 7 post-pellet implantation reversed morphine-induced thermal hypersensitivity with peak effect observed at 2 hours, and drug effect dissipating within 2.5 hours. B) ICR mice developed tactile hypersensitivity within 7 days of pellet implantation (Pre-AMG). Oral administration of AMG 0347 (3 mg/kg) on day 7 post-pellet implantation reversed morphine induced tactile hypersensitivity with peak effect observed at 2 hours, and drug effect dissipating within 2.5 hours. Paw-withdrawal responses in placebo treated animals were not different from baseline values throughout the time course. * indicates significant difference from baseline ($p < 0.05$).

Fig. 3.

A) Rats developed morphine-induced thermal hypersensitivity, measured using the Hargreaves paw-flick test, within 6 days of pellet implantation (Pre-AMG). Oral administration of AMG 0347 (3 mg/kg) on day 6 post-pellet implantation reversed morphine-induced thermal hypersensitivity with peak effect observed at 2 hours, and drug effect dissipating within 2.5 hours. B) Rats developed tactile hypersensitivity within 6 days of pellet implantation (Pre-AMG). Oral administration of AMG 0347 (3 mg/kg) on day 6 post-pellet implantation reversed morphine induced tactile hypersensitivity with peak effect observed at 2 hours, and drug effect dissipating within 2.5 hours. Paw-withdrawal responses in placebo treated animals were not different from baseline values throughout the time course. * indicates significant difference from baseline ($p < 0.05$).

Fig. 4.

A) Immunohistochemical staining was performed on DRG tissue collected 6 days after morphine or placebo pellet implantation. Sustained morphine across 6 days, but not 2 days, increased immunofluorescent staining for TRPV1 positive profiles. B) Cell counts verified that there is a small (13%) but significant increase in the percentage of TRPV1-ir positive cell bodies within the DRG. * indicates significant difference from placebo ($p < 0.05$) C) Immunofluorescent labeling in the spinal dorsal horn of morphine or placebo treated rats 6 days after pellet implantation.

Fig 5.

A) Sustained morphine across 6 days doubled the number of capsaicin-induced flinching compared to placebo controls. B) Rats receiving morphine infusion showed an approximate 5-fold increase in plasma extravasation compared to placebo treated rats. * indicates significant difference from placebo ($p < 0.05$)