Published in final edited form as:

Brain Res. 2010 October 28; 1358: 64–70. doi:10.1016/j.brainres.2010.08.032.

Opioid-induced latent sensitization in a model of non-inflammatory viscerosomatic hypersensitivity

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Abstract

Exposure to opioids can induce a state of “latent sensitization” characterized by long-lasting enhanced responses to subsequent cutaneous injury. Here, we explored the possibility that prior treatment with morphine could induce a state of latent sensitization to visceral pain conditions. Following butyrate enemas to induce non-inflammatory visceral pain, acute morphine administration produced dose-related inhibition of referred viscerosomatic hypersensitivity. Treatment with morphine for a period of six days resulted in a persistent hyperalgesia that resolved many days after termination of drug administration. In morphine pre-exposed rats, butyrate-induced referred hypersensitivity was enhanced and extended in duration. No differences were observed in the morphine dose-response curve in suppression of acute nociception (i.e., the hot-plate assay) when morphine pre-exposed rats were compared to naïve rats indicating that opioid antinociceptive tolerance was not present. However, the morphine dose-response curve to suppress evoked viscerosomatic hypersensitivity was displaced to the right by approximately 4-fold in morphine pre-exposed rats. Induction of viscerosomatic hypersensitivity resulted in an increased labeling of CGRP-, but not substance P-positive cells in the lumbar dorsal root ganglia; increased labeling was not affected by prior exposure to morphine. The data indicate that a period of morphine exposure can induce a state of “latent-sensitization” to subsequent visceral pain characterized by extended duration of hypersensitivity. This condition likely reflects enhanced visceral “pain” intensity as a consequence of persistent pronociceptive adaptive changes.

Keywords

IBS; opioid-induced latent sensitization

1. INTRODUCTION

Opioids are the most potent analgesics currently available, and are widely used to treat severe acute pain, cancer pain and have emerging use in the treatment of chronic non-

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malignant pain. Opioid use is sometimes associated with analgesic tolerance that can occur within days and requires increased dosing to maintain desired analgesic effects (Dogrul et al., 2003; Hanks et al., 2001; Rivat et al., 2002; Sim et al., 2007). There is a growing awareness that exposure to opioids can cause the development of opioid-induced hyperalgesia in preclinical models and possibly in humans (Angst and Clark, 2006; Koppert et al., 2003). For example, clinical studies with volunteers undergoing surgery revealed that pre-operative opioid administration can result in increased post-operative pain and an increased need for analgesics (Hood et al., 2003). Clinical studies revealed that former opioid addicts on methadone maintenance therapy exhibited decreased pain tolerance in a cold-pressor test (Compton et al., 2001). Opioid-induced hyperalgesia has been demonstrated in numerous animal studies (Celerier et al., 2000; Laulin et al., 1998; Laulin et al., 1999; Ossipov et al., 2004; Vanderah et al., 2001). Animals that received subcutaneous or intrathecal infusions of morphine developed behavioral signs of thermal hyperalgesia and tactile allodynia while the opioid was still being administered (Gardell et al., 2002; Vanderah et al., 2001). Likewise, individuals undergoing methadone maintenance therapy demonstrated hyperalgesia and cold allodynia while still taking methadone (Athanasos et al., 2006; Doverty et al., 2001).

More recent findings suggest that previous exposure to opioids for some period of time results in a long lasting increase in the response to a subsequent injury (e.g., a surgical procedure) or to normally non-noxious or noxious stimuli applied to cutaneous tissues (i.e., hyperalgesia). This phenomenon persists long after cessation of the period of opioid administration, and has been termed “latent pain sensitization” (Celerier et al., 2001a). The main features of opioid-induced latent sensitization are (1) increased intensity and extended duration of pain and (2) reduced responsiveness to the analgesic effects of pain management drugs. Patients that have had previous exposure to opioids can exhibit an increased duration and intensity of pain in multiple conditions, such as migraine headache (Jakubowski et al., 2005), sphincter of Oddi dysfunction (Freeman et al., 2007), post-operative pain (Rapp et al., 1995; Sim et al., 2007) or during childbirth (Meyer et al., 2007) when compared to opiate naive patients. Prior exposure of rats to morphine infusion resulted in pain hypersensitivity in a model of surgical incision, even though baseline sensory thresholds had returned to normal values (Rivat et al., 2009). Although the mechanism underlying this phenomenon remains unclear, it has been proposed that opioids can elicit neuroplastic adaptations following some period of exposure (Gardell et al., 2002; Gardell et al., 2003; Okada-Ogawa et al., 2009). These neuroplastic changes persist even after behavioral signs of enhanced abnormal pain have resolved, and might underlie heightened nociception in response to an additional insult (Laboureyras et al., 2009; Ossipov et al., 2003; Ossipov et al., 2004; Simonnet, 2009).

Based on these observations, we have determined whether the “latent sensitization” could also be demonstrated in other pain states with potential clinical relevance. We have used an established animal model thought to be representative of irritable bowel syndrome (IBS), where colonic hypersensitivity is brought about by intracolonic injections of sodium butyrate (Bourdu et al., 2005). This treatment induces robust visceral and referred cutaneous hypersensitivity within 3 days post-treatment without inflammation-induced mucosal damage in the colon.

2. RESULTS

Morphine reverses non-inflammatory referred visceral hypersensitivity

Intra-colonic administration of butyrate produced viscerosomatic hypersensitivity, as indicated by a significant ($p < 0.05$) reduction in mean withdrawal threshold to 0.3 ± 0.05 g from a pre-treatment baseline value of 6 g when von Frey filaments were applied to the back of the rats, corresponding to lumbar dermatomes. Morphine produced dose-dependent reversal of viscerosomatic hypersensitivity, indicated by an A_{50} (95% C.L.) of 7.7 mg/kg (1.5 - 38.2 mg/kg) (Figure 1).

Rats previously exposed to morphine do not show antinociceptive tolerance 14 days after pump removal

The rats implanted with morphine pumps displayed morphine-induced antinociception six hours after pump implantation, as indicated by a significant ($p < 0.05$) increase in hot-plate latency to 16.3 ± 0.8 sec from a baseline mean of 11.2 ± 0.6 sec (Figure 2A). On day 6 after pump implantation, morphine-induced hyperalgesia was indicated by a significant ($p < 0.05$) reduction in hot-plate latency to 8.2 ± 0.4 sec (Figure 2A), and resolution of hyperalgesia was indicated by a normalized hot-plate latency of 12.0 ± 0.9 sec on day 20 (Figure 2A). Saline-infused rats did not show significant changes in hot-plate latency over this time period (Figure 2A). At day 20 (14 days after removal of the pumps), dose-response curves for morphine in the hot-plate test were generated with saline-treated and morphine-treated groups. Morphine produced equivalent antinociceptive effects in both groups (Figure 2B.). The A_{50} (95% C.L.) for the saline-infused group, 3.4 mg/kg (0.4 - 8.3 mg/kg), was not significantly ($p > 0.05$) different from that for the morphine-infused group, which was 3.6 mg/kg (0.6 - 4.8 mg/kg). These data indicate that the antinociceptive tolerance to morphine has resolved by 2 weeks after termination of the previous morphine exposure.

Morphine pre-exposure shows enhanced hypersensitivity and delayed recovery from butyrate

We determined whether prior exposure to morphine infusion could alter characteristics of non-inflammatory visceral pain. Animals received butyrate enemas (1 mL sodium butyrate solution twice daily for 3 days) to produce referred cutaneous hypersensitivity to tactile stimuli applied to the lumbar dermatomes of the back of the animals. Both saline and morphine pre-exposed rats developed viscerosomatic hypersensitivity to the application of von Frey filaments within 6 days of receiving the butyrate administration (Fig. 3). Saline treated animals showed recovery with tactile sensory thresholds returning to baseline by 10-11 days post treatment. In contrast, the response thresholds of morphine pre-exposed rats were significantly ($p < 0.05$) lower than those of saline-treated rats and did not return to pre-treatment baseline values until 17 days after the butyrate administration (Fig. 3). Thus, prior exposure to morphine extends the duration of butyrate induced hypersensitivity.

Morphine dose-response curves were generated in both the saline-treated group and the morphine-treated group. The morphine dose-response curve for the saline-infused group was similar (Fig. 4) to that seen in naïve, untreated animals shown in Fig. 1. The A_{50} (95% C.L.) value for the saline-treated group was 5.4 mg/kg (3.6 - 8.1 mg/kg). In contrast, the

morphine-pretreated group demonstrated a 4-fold shift to the right, indicated by an A_{50} (95% C.L.) value of 21.8 mg/kg (6.7 – 71 mg/kg) (Figure 4). Furthermore, although morphine produced 100% reversal of tactile hypersensitivity in the non-infused and saline-infused groups, the maximum dose tested, 30 mg/kg, produced only a $62.4 \pm 5.2\%$ anti-allodynic effect (Figure 4). These results indicate that pre-exposure to morphine was associated with a reduced antinociceptive effect of a second morphine exposure suggesting an enhanced intensity of the viscerosomatic hypersensitivity.

Labeling of CGRP, but not of substance P, is enhanced in the lumbosacral DRGs of IBS rats

In order to examine the possible changes in expression of CGRP and substance P in the hypersensitivity induced by the butyrate enemas, the lumbosacral DRG of rats with butyrate administration or saline treatment were dissected 6 days after butyrate injection and immuno-labeled for CGRP and substance P. The proportion of DRG profiles labeled for CGRP in DRG taken from the saline-infused group was $35.6 \pm 1.3\%$ whereas that from the butyrate-treated group was significantly ($p < 0.05$) increased to $53.5 \pm 1.6\%$ (Figure 5). In contrast, no differences were observed in the proportion of DRG profiles that were labeled for substance P (Figure 5). These values were $19.4 \pm 0.9\%$ and $19.1 \pm 1.4\%$, respectively.

CGRP and substance P expression levels are not affected by the previous exposure to morphine in rats

Rats previously implanted with either morphine or saline minipumps presented thermal latencies similar to baseline on day 14 after removal of the minipumps. These rats were then injected with sodium butyrate or saline as described before. They were divided into four groups : (Morphine infusion)+saline, (Morphine infusion)+butyrate, (Saline infusion) +saline, (Saline infusion)+butyrate. On day 6 after butyrate injection, the lumbosacral DRGs were dissected and immunostained to investigate the number of cells positively labeled for either CGRP or substance P. There was no statistical difference in substance P positive cells expression level among these four groups (Figure 6). For CGRP, either morphine pre-exposed or saline pre-exposed rats had a significantly increased expression level compared to their respective vehicle group (49.5% vs 38.2 ; 50.3% vs 38.0% , $P < 0.05$ respectively). The increase in CGRP expression in rats with previous exposure to morphine was not greater than the increase observed in rats previously implanted with saline pumps (Figure 6). These results suggest that previous exposure to morphine does not produce any further increases in expression of CGRP or substance P.

3. DISCUSSION

The present study has employed a rodent model of non-inflammatory visceral pain to demonstrate that (a) systemic administration of morphine produces dose-dependent blockade of referred viscerosomatic cutaneous hypersensitivity; (b) previous exposure to morphine can induce latent sensitization that is manifested by an extended duration and intensity of butyrate-induced referred cutaneous hypersensitivity and (c) there is a diminished response to acute morphine treatment in previously morphine-exposed rats that is not explained by opiate tolerance. In addition, treatment with butyrate increased the number of cells labeled

for pronociceptive transmitters in the lumbosacral DRG and prior exposure to morphine did not further enhance cells positively labeled for CGRP or substance P in lumbosacral DRGs.

Opioid receptors are found throughout the nervous system, including the peripheral and visceral nerves, and are present on afferent fibers that transmit nociceptive inputs into the spinal cord as well as on second- and third-order neurons that transmit these signals to higher centers (Hammerle and Surawicz, 2008; Quirion, 1984; Stein et al., 2001). Thus, opioids acting at either peripheral or central sites can inhibit nociceptive transmission from visceral origin. Moreover, visceral afferents overlap with the projection targets of somatic afferent fibers, and explain in part the referral of visceral pain to somatic sites (Brumovsky and Gebhart, 2009; Cervero, 1991; Gebhart and Ness, 1991). In the present investigation, we demonstrated that morphine produced dose-dependent antinociception against hypersensitivity induced in an animal model thought to be relevant to IBS. This result is consistent with observations made in both preclinical animal models of visceral pain as well as in clinical reports with patients with bowel pain from conditions including IBS (Camilleri, 2004; Hammerle and Surawicz, 2008).

In the present study, systemic morphine infusion produced thermal hyperalgesia in rats on day 6, which is consistent with observations from our laboratory and from others (Gardell et al., 2002; King et al., 2005b; Vanderah et al., 2001). Importantly, thermal hyperalgesia developed while morphine was still being administered, precluding the possibility that hyperalgesia was a consequence of opioid withdrawal. Hyperalgesia resolved 14 days after the pumps were removed, as indicated by normalized behavioral responses. Moreover, antinociceptive tolerance to morphine was not present at that time since the dose-response curves for both saline-treated and morphine-treated rats in somatic pain were similar. However, the introduction of a second “injury”, in the form of colonic hypersensitivity induced by the butyrate enemas, unmasked a state of latent sensitization. Latent sensitization was indicated by the enhanced intensity and duration of butyrate-induced viscerosomatic hypersensitivity in the morphine-exposed rats as well as the diminished antinociceptive potency of morphine. The dose-response curve for morphine after the intracolonic butyrate administration was shifted 4-fold to the right, and the maximal response to morphine at the highest dose was significantly less in rats with prior morphine exposure. In addition, previous exposure to morphine infusion delayed recovery of the hypersensitivity induced by butyrate enemas. These results indicate a long-term change in pain processing that persists long after removal of the opioid, and recovery from opioid induced hyperalgesia (i.e. return to “normal” sensory thresholds). This finding is consistent with those studies showing enhanced responses to nociceptive stimuli after termination of opioid administration (Celerier et al., 2000; Celerier et al., 2001a; Celerier et al., 2001b). Moreover, the present study extends the observations of development of latent sensitization to include visceral pain.

We have previously shown that prolonged exposure to morphine increases the expression of CGRP in the DRG and spinal dorsal horn (Gardell et al., 2003), as well as that of substance P and of the NK1 receptor (King et al., 2005a). Although these changes may account in part for opioid-induced hyperalgesia, a possible contribution to latent sensitization is not certain. In the present investigation, exposure to intracolonic butyrate increased expression of CGRP

but not substance P. However, prolonged exposure to morphine did not produce any changes in CGRP or substance P expression in DRG that was still evident 14 days after termination of the opioids infusion, even in animals that received intracolonic butyrate.

The basis for latent sensitization remains unknown but appear unrelated to changes in the expression of pronociceptive transmitters in the DRG. One possibility may be that opioids elicit neuroplastic adaptations in descending pain modulatory systems. Ablation of dorsal horn projection neurons that express the NK1 receptor has been shown to abolish descending pain facilitation from the rostroventral medial medulla (RVM), presumably by interrupting a spinal/supraspinal pain facilitatory loop (Suzuki et al., 2002). Recent studies demonstrated that ablation of these projection neurons also abolished latent sensitization to surgical incision in rats previously exposed to opioids (Rivat et al., 2009). Latent sensitization in the same study was blocked by microinjection of lidocaine into the RVM (Rivat et al., 2009). It was also shown that prolonged morphine exposure caused a loss of diffuse noxious inhibitory controls (DNIC) that was restored by blockade of RVM activity with lidocaine (Okada-Ogawa et al., 2009). It was interpreted that prolonged opioids exposure could promote nociception by reducing descending pain inhibitory systems, though enhanced facilitation cannot be ruled out (Okada-Ogawa et al., 2009).

In summary, the present investigation demonstrates that prolonged exposure to morphine can cause latent sensitization that can be expressed in a model of visceral pain even when no inflammation is present. The results presented here suggest the need for further clinical evaluation of opioid use for the treatment of visceral pain states such as IBS in order to determine whether periods of prolonged use might contribute to long-lasting enhanced sensitivity to visceral pain.

4. METHODS AND MATERIALS

Animals

Adult, male Sprague Dawley rats (Harlan, Indianapolis, IN), weighing 150–200 g, were maintained in a climate-controlled room with ad libitum food and water on a 12-hour light/dark cycle (lights on at 07:00 hours). All procedures followed the policies of the International Association for the Study of Pain and the National Institutes of Health (NIH) guidelines for the handling and use of laboratory animals. Studies were approved by the Institutional Animal Care and Use Committee of the University of Arizona. All measurements were made by an investigator blinded to the experimental treatments.

Viscerosomatic Hypersensitivity Model

Male Sprague-Dawley rats weighing 175–200 g received intracolonic injections of a sodium butyrate solution (110 mg/ml, saline vehicle) twice daily for 3 days. Rats were fasted for 12 hours before the initiation of the injections to allow for a clear colon. For each injection, a catheter made of PE-100 polyethylene tube was placed into the colon at 7 cm from the anal opening and the rats received a 1 ml of the sodium butyrate solution at neutral pH. Care was taken to avoid damage of the colonic wall by insertion of the catheter.

Behavioral Testing

Referred viscerosomatic hypersensitivity in the colonic hypersensitivity model was quantified by applying von Frey filaments to the lumbar dermatomes on the back of the rats. The backs of the rats, corresponding to the lumbar dermatomes, were shaved before any manipulation and the rats were acclimated inside Plexiglas boxes for 30 minutes on the day of testing. Calibrated von Frey filaments, ranging from 0.04 g to 6 g, were applied to the back 5 times for 1 sec. The mechanical threshold corresponded to the force of the von Frey filament that induced wrinkling of the skin which was followed by escape behavior from the filament.

Morphine pre-exposure process and inducement of latent sensitization

Male Sprague Dawley rats weighing 175-200 g were implanted subcutaneously with osmotic minipumps (Alza, Mountain View CA, model 2001) with a nominal flow rate of 1 μ l per hour and duration of 7 days and delivering either normal saline or morphine (64 mg/kg/day). Before pump implantation, baseline nociceptive responses to the 52°C hot-plate were determined and then again 6 hrs after implantation of the minipumps in order to confirm the presence of morphine-induced antinociception. On the 6th day after pump implantation, rats underwent thermal testing again to confirm the presence of morphine-induced hyperalgesia. The pumps were then removed and the rats left to recover for 14 days afterwards. On day 14 (i.e.; day 20 after pump implantation), rats underwent thermal testing to confirm a return to baseline latencies. If rats presented with “baseline” thermal latencies (90% of rats showed baseline sensory thresholds on day 20), they then received sodium butyrate to induce colonic hypersensitivity as described. On the 6th day after initiation of the butyrate injections, rats were tested for referred viscerosomatic hypersensitivity as before. The time course of the hypersensitivity was followed and compared to rats previously implanted with saline pumps.

Tissue Preparation

On the 6th day after receiving intracolonic butyrate treatments, rats were anesthetized with an overdose of ketamine and transcardially perfused with PBS followed by 4% paraformaldehyde. Lumbosacral (i.e., L6, S1 and S2) dorsal root ganglia (DRG) were dissected. Tissues were fixed overnight in 4% paraformaldehyde, cryoprotected in 30% sucrose solution overnight, embed with OCT compound and sectioned with a cryostat (10 μ m/each section) at -20°C. Sections were attached on the gelatin-covered glass slides.

Staining Procedures

Primary antisera were rabbit anti-CGRP (1:5,000 Peninsula), Anti-substance P (1:5,000 Chemicon/Millipore). Secondary antisera were Cy3-conjugated anti-rabbit IgG (1:500 Jackson Labs), Alexa Fluor® 488 goat anti-rabbit IgG (1:1,000 Invitrogen). Sections were washed 5 times (5 minutes/per wash) with PBS, then incubated with 5 % NGS (goat serum) in PBS with 1 μ l of triton X-100 for 1 hour. After that, sections were incubated with primary antibody of interest in 2% NGS/PBS/triton X-100 overnight. The next day sections were washed with PBS 5 times (10 min/per wash), and then incubated in the secondary antibody for two hours. Afterwards, they were washed 5 times (5 min/per wash) then dried in a dark

box for 30 min before being sealed with mounting medium. The images were acquired using a Nikon E800 fluorescent microscope with a Hamatsu C5810 color CCD camera and Wasabi software (Hamatsu Photonic System Inc., San Jose, CA). Cells were counted by an observed blinded to the treatment. The number of neurons counted ranged from 126 to 205 profiles per section. A total of 3 rats were used per treatment and 5 sections were obtained from each rat.

Statistical Analysis

All data were expressed as mean $\pm$ SEM. Data were analyzed using a one-factor analysis of variance (ANOVA) followed by a Tukey's Multiple Comparison Post Hoc Test to detect significant differences in behavioral outcomes within each experimental group over time. A Student t test was used to compare two treatment groups. Significance was established at $P < 0.05$. Dose-response curves were generated by converting data to % maximal possible effect (%MPE) by the formula $100 \times (\text{test-baseline})/(\text{cut-off} - \text{baseline})$, where cut-off is 6 g for tactile responses and 30 sec for hot-plate latencies. The A_{50} and 95% confidence intervals were determined by regression analyses of the log dose-response curve with JFlashcalc (www.u.arizona.edu/~michaelo).

Abbreviations

CGRP	Calcitonin gene-related peptide
DRG	Dorsal root ganglion
IBS	Irritable bowel syndrome
MPE	Maximum possible effect
RVM	Rostral ventromedial medulla

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Research Highlights

- Morphine exposure enhances sensitivity to light touch in a model of visceral pain.
- Morphine exposure sensitizes rats to subsequent butyrate challenge.
- Morphine exposure increases CGRP in primary afferent neurons.
- Increased CGRP underlies opioid-induced latent sensitization.

Figure 1.

Morphine produces dose-dependent anti-allodynia in an animal model of experimental IBS. Rats received morphine (1, 3, 6, and 10 mg/kg, i.v.) after receiving intracolonic butyrate solution (110 mg/kg). Withdrawal thresholds to von Frey filaments were determined 30 minutes after morphine administration. (n=6 per group).

Figure 2.

Effect of previous sustained infusion of morphine in rats. **A.** Rats received infusions of morphine (64 mg/kg/day) or saline and were subjected to the 52°C hot-plate test. Hot-plate latencies were determined before minipump implantation, 6 hours after implantation, day 6 after implantation and day 20 after implantation (i.e.; 14 days after pump removal). # $P < 0.05$ compared to baseline, * $P < 0.05$ compared to saline group. **B.** Dose-response curve for systemic morphine is shown for saline-treated and morphine-treated groups in the hotplate test 14 days after removal of the minipumps. (n=6-14 per group)

Figure 3.

Rats that had previously received morphine or saline infusion were tested 20 days post minipump implantation and showed no hypersensitivity during baseline. Butyrate enemas produced referred viscerosomatic cutaneous hypersensitivity within 6 days of injection. Saline treated rats returned to baseline sensory thresholds by 11 days post treatment. In contrast, morphine treated rats consistently showed cutaneous hypersensitivity throughout the testing period. (n=6 per group).

Figure 4.

Dose-dependent effect of systemic morphine in rats pre-exposed to saline or morphine infusions and then challenged with intracolonic administration of butyrate 14 days after pump removal. Rats received morphine (1, 3, 6, 10, 30 mg/kg) and were tested with von Frey filaments 30 minutes after injection. (n=6 per group)

Figure 5.

Percentage of neurons positively labeled for CGRP or substance P among the total counted neurons in the lumbar DRGs from rats treated with intracolonic saline or butyrate. * P<0.05 compared to saline treated group.

Figure 6.

Percentage of neurons positively labeled for CGRP or substance P expression among the total counted neurons in the lumbar DRGs from rats that were pre-treated with saline or morphine infusion and then challenged 14 days after minipump removal with intracolonic saline or butyrate. * $P < 0.05$ compared to saline treated group.