Peripheral Nerve Injury Reduces Analgesic Effects of Systemic Morphine *via* Spinal 5-Hydroxytryptamine 3 Receptors

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ABSTRACT

Background: Morphine produces powerful analgesic effects against acute pain, but it is not effective against neuropathic pain, and the mechanisms underlying this reduced efficacy remain unclear. Here, the authors compared the efficacy of systemic morphine between normal rats and rats with peripheral nerve injury, with a specific focus on descending serotonergic mechanisms.

Methods: After L5 spinal nerve ligation injury, male Sprague–Dawley rats were subjected to behavioral testing, *in vivo* microdialysis of the spinal dorsal horn to determine serotonin (5-hydroxytryptamine [5-HT]) and noradrenaline release, and immunohistochemistry (n = 6 in each group).

Results: Intraperitoneal administration of morphine (1, 3, or 10 mg/kg) produced analgesic effects in normal and spinal nerve ligation rats, but the effects were greater in normal rats ($P < 0.001$). Morphine increased 5-HT release (450 to 500% of the baseline), but not noradrenaline release, in the spinal dorsal horn *via* activation of serotonergic neurons in the rostral ventromedial medulla. Intrathecal pretreatment with ondansetron (3 μg), a 5-HT3 receptor antagonist, or 5,7-dihydroxytryptamine creatinine sulfate (100 μg), a selective neurotoxin for serotonergic terminals, attenuated the analgesic effect of morphine (10 mg/kg) in normal rats but increased the analgesic effect of morphine in spinal nerve ligation rats (both $P < 0.05$).

Conclusions: Systemic administration of morphine increases 5-HT levels in the spinal cord, and the increase in 5-HT contributes to morphine-induced analgesia in the normal state but attenuates that in neuropathic pain through spinal 5-HT3 receptors. The plasticity of the descending serotonergic system may contribute to the reduced efficacy of systemic morphine in neuropathic pain. **(Anesthesiology 2014; 121:362-71)**

THE analgesic effects of opioids such as morphine are thought to arise from activation of μ-opioid receptors in neurons in the brain, medulla, spinal cord dorsal horn, and peripheral terminals of primary afferents.[1,2] The analgesic actions of systemic morphine are in part mediated by periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM) that exert a net inhibitory effect on nociceptive processing in the spinal dorsal horn.[1] The role of the RVM in the modulation of nociceptive inputs has been extensively studied, and this region has been characterized as an important source of descending modulatory inputs that both inhibit and enhance the perception of pain at the level of the spinal cord.[1,3,4] When administered into the RVM, opioids directly inhibit on-cells and indirectly stimulate off-cells, thus inhibiting spinal nociresponsive neurons and behavioral responses to noxious stimuli.[5] The RVM also includes the raphe magna, which contains serotonergic neurons that project to the spinal cord.[6] Systemic administration of morphine may increase serotonin (5-hydroxytryptamine [5-HT]) levels in the spinal cord,[7–9] thus inhibiting the transmission of nociceptive information.[10,11] However, it has been suggested that descending facilitation from the RVM, including its serotonergic neurons, may play an essential role in maintaining neuropathic pain.[12–15] Although the exact mechanism underlying the descending facilitation remains unclear, activation of spinal 5-HT3 receptors is considered critical.[4,13,16]

What We Already Know about This Topic

- Opioids are a mainstay of treatment for many types of pain but may be less effective against neuropathic pain

What This Article Tells Us That Is New

- Using a rat spinal nerve ligation model, the authors observed that neuropathic rats were less sensitive to morphine than normal animals
- The enhanced spinal release of serotonin acting through 5-hydroxytryptamine 3 receptors may be responsible for the reduced effects of morphine

Neuropathic pain is a chronic and persistent pain that is difficult to treat with conventional analgesics, and it is characterized by altered pain perception including enhanced sensitivity to noxious stimuli (hyperalgesia) and abnormal sensitivity to previously nonpainful stimuli (allodynia). Morphine produces powerful analgesic effects in acute pain, but its efficacy is reduced in neuropathic pain.[17–19] We hypothesized that increased 5-HT levels in the spinal cord contribute to the reduced efficacy of systemically administered morphine in neuropathic pain *via* spinal 5-HT3 receptors. The current

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study was undertaken to compare the efficacy of systemic morphine between normal rats and rats with peripheral nerve injury with particular regard to the function of descending inhibitory systems including serotonergic mechanisms.

Materials and Methods

Surgical Preparation

The study was approved by the Animal Care and Use Committee of the Gunma University School of Medicine (Maebashi, Japan 12–058). Male Sprague–Dawley rats (250 g, n = 156; SLC, Shizuoka, Japan) were used in all experiments. No data were lost to observation during the experiments. The animals were housed under a 12-h light–dark cycle, with food and water available *ad libitum*. Spinal nerve ligation (SNL) was performed as previously described.[20] The animals were anesthetized with inhaled isoflurane in oxygen, and the right L5 spinal nerve was tightly ligated with 5-0 silk and cut just distal to the ligature. Normal (unoperated) rats were used as a control group in all experiments; the normal rats did not undergo any aspect of the SNL procedure (*i.e.*, skin preparation, incision, tissue dissection, or nerve ligation) but were housed and handled similarly to the SNL rats. For SNL rats, experiments were performed 2 to 3 weeks after nerve injury to permit the full development of behavioral sensitivity and pathological changes reflective of neuropathic pain.

Behavioral Testing

The withdrawal threshold to pressure applied to the hind paw, expressed in grams, was measured using an analgesimeter (37215, Ugo Basile, Comerio, Italy) as previously described.[21] The device applies increasing pressure to the hind paw. When the animal withdraws the paw, the pressure is immediately released, and the nociceptive threshold is measured in grams. A cutoff of 250 g was used to prevent potential tissue injury. All animals were trained for 3 days with the device before baseline values were recorded. Experiments with normal and SNL rats were performed separately. The experimenter was unaware of drug and dose.

Drug Administration

Normal animals and SNL rats were intraperitoneally injected with morphine (1, 3, or 10 mg/kg). Antagonist studies were performed using the 5-HT3 receptor antagonist, ondansetron (0.3 or 3 μg). Saline or the antagonist solution was administered intrathecally 15 min before morphine injection. The ondansetron doses were selected according to previous studies[16] and our preliminary studies. For intrathecal administration, ondansetron was dissolved in 5 μl of saline and injected into the L5 to L6 intervertebral space using a 30-gauge needle. For intraperitoneal administration, morphine was dissolved in 0.5 ml of saline.

To examine the role of 5-HT in the spinal cord on morphine-induced analgesia, 5,7-dihydroxytryptamine (5,7-DHT) creatinine sulfate (100 μg in 10 μl), a selective neurotoxin for serotonergic terminals, was administered intrathecally to normal rats and SNL rats 2 weeks after nerve ligation. To prevent damage to noradrenergic neurons, we pretreated the animals with desipramine (20 mg/kg, intraperitoneal), an inhibitor of neuronal noradrenaline reuptake[22] 30 min before 5,7-DHT (or saline) administration. Ten days later, behavioral experiments were performed. In preliminary experiments to confirm that treatment with 5,7-DHT and desipramine selectively ablated serotonergic terminals, we used our previously described homogenate preparation[23] to determine that the 5-HT concentration in the spinal cord was decreased (1.21 ± 0.12 pg/mg after saline and 0.23 ± 0.41 pg/mg after 5,7-DHT, respectively, n = 6 in each group) at 10 days after administration (the time point where behavioral effects were performed), whereas the noradrenaline concentration was not affected (0.49 ± 0.08 pg/mg after saline and 0.47 ± 0.62 pg/mg after 5,7-DHT, respectively, n = 6 in each group). Morphine, ondansetron, 5,7-DHT, and desipramine were purchased from Sigma Co. (St. Louis, MO).

Microdialysis Studies

Microdialysis studies were performed to measure noradrenaline and 5-HT levels in the spinal dorsal horn of normal rats or SNL rats according to a previously described protocol.[23–25] Anesthesia was induced using urethane (1.2 to 1.5 g/kg, intraperitoneally) and maintained with 0.5% isoflurane in 100% oxygen through a nose cone. A 24-gauge indwelling catheter was placed in the peritoneal cavity for drug administration. The left femoral vein was cannulated for saline infusion (1 ml/h), rectal temperature was maintained at 37° to 38°C, and the L3 to L6 level of the spinal cord was exposed. The microdialysis probe (outer diameter = 0.22 mm, inner diameter = 0.20 mm, length = 1 mm; A-I-8-01; Eicom Co., Kyoto, Japan) was inserted from just lateral to the dorsal root and advanced at a 20° angle to a depth of 1 mm and perfused with Ringer's solution at a constant flow rate (1 μl/min). After 120 min of constant perfusion, two consecutive samples were collected to determine the basal noradrenaline and 5-HT concentrations in the dialysate. Saline (0.5 ml) or morphine (10 mg/kg) was administered intraperitoneally to the rat through an indwelling catheter, and 15-min perfusate fractions were collected into an autoinjector (EAS-20; Eicom Co.). Samples (15 μl) were automatically injected and analyzed for noradrenaline and 5-HT concentration using high-performance liquid chromatography with electrochemical detection by an HTEC-500 analyzing system (Eicom Co.). The sample was then separated on the column (2.0 mm × 200 mm, EICOMPAC CAX; Eicom Co.) using the mobile phase that consisted of 0.1 M ammonium acetate buffer (pH 6.0) and methanol (7:3 v/v) containing 0.05 M sodium sulfonate and 50 mg/l EDTA-2Na. The detection limit of this assay is 0.1 pg per injection (information from Eicom Co.*).

*Available at: http://www.eicom.co.jp/ECD_application.html. Accessed March 2, 2014.

Immunohistochemistry

To determine whether systemic administration of morphine activates serotonergic neurons in the RVM, we performed immunohistochemistry using antibodies for tryptophan hydroxylase (an enzyme responsible for 5-HT synthesis) and c-Fos (a marker for neuronal activation). Normal animals and SNL rats were deeply anesthetized with intraperitoneal pentobarbital (100 mg/kg) 2 h after intraperitoneal administration of morphine (10 mg/kg) or saline and perfused through the aorta with 4°C 0.01 M phosphate-buffered saline containing 1% sodium nitrite, followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline. The brain was dissected out, postfixed in the same fixative for 3 h, and cryoprotected by immersion in 0.1 M phosphate buffer containing 30% sucrose at 4°C. Three days later, the brain containing the RVM was cut transversely into 30-μm sections using a cryostat and the sections were mounted on glass slides.

The mounted sections were rinsed two times with 0.01 M phosphate-buffered saline containing 0.3% Triton X-100. After pretreatment with 1.5% normal goat serum (S-1000; Vector Laboratories, Inc., Burlingame, CA), the sections were incubated for 24 h at 4°C with a mouse monoclonal anti-tryptophan hydroxylase antibody (1:1,000, MAB5278; Chemicon International Inc., Temecula, CA) and a rabbit monoclonal anti-c-Fos antibody (1:1,000, sc-253; Santa Cruz Biotechnology Inc., Santa Cruz, CA) in 1.5% normal goat serum. The sections were then rinsed twice with 0.01 M phosphate-buffered saline containing 0.3% Triton X-100 and incubated for 1 h with an AlexaFluor 488–conjugated goat anti-mouse secondary antibody (1:100; Invitrogen, Cergy Pontoise, France) in 1.5% normal goat serum to label serotonergic neurons and an AlexaFluor 568–conjugated goat anti-rabbit secondary antibody (1:100; Invitrogen) in 1.5% normal goat serum to label c-Fos. Images were captured on an Olympus FSX100 microscope (Olympus Co., Tokyo, Japan). The number of c-Fos–positive serotonergic neurons in the RVM was compared between normal animals and SNL rats.

Statistics

We selected a sample size of 6 based on a previous study.[25] The statistical analysis was conducted using SigmaPlot 12 (Systat Software Inc., San Jose, CA). Data were normally distributed (Shapiro–Wilk test) and are presented as the mean ± SD. All statistical comparisons involved a two-tailed hypothesis of either an increase or a decrease in the measurement variable. Time-course data from the behavioral and microdialysis studies were analyzed using a two-way repeated-measures ANOVA. When significant differences were observed, Student–Newman–Keuls *post hoc* tests were performed for between-group comparisons and comparisons at each time point. The paired *t* test was also used for some analyses in behavioral studies. For comparisons of behavioral data between normal and SNL rats, the area under the time-course curve for the percentages of the maximum possible effect was calculated from individual scores at each time point using the trapezoidal rule over the 240-min observation period: percentage of the maximum possible effect = (postdrug threshold − predrug threshold) × 100/(250 g − predrug threshold). The area under the time-course curve and immunohistochemical data are presented as the mean ± SD and were analyzed by one-way ANOVA, followed by Student–Newman–Keuls *post hoc* tests. *P* value less than 0.05 was considered statistically significant.

Results

Behavioral Studies

Intraperitoneal administration of morphine (1, 3, and 10 mg/kg) produced analgesic effects in normal and SNL rats (n = 6 in each group, $P < 0.001$ and $P = 0.008$, respectively; fig. 1, A and B). Withdrawal thresholds were higher in normal rats treated with 3 and 10 mg/kg morphine than in normal saline-treated rats ($P < 0.001$ and $P < 0.001$, respectively; fig. 1A). In contrast, withdrawal thresholds were higher only in SNL rats treated with 10 mg/kg morphine when compared with saline-treated SNL rats ($P = 0.01$; fig. 1B). Peak behavioral effects were observed 30 to 60 min and 15 min after morphine administration in the normal and SNL rats, respectively. The duration of the effect was longer in normal rats than in SNL rats (240 *vs.* 90 min). Based on the area under the time-course curve data, the effects of 3 and 10 mg/kg morphine were greater in normal rats than in SNL rats (n = 6 in each group, $P < 0.001$ and $P < 0.001$, respectively; fig. 1C). On the basis of these results, we selected the morphine dose of 10 mg/kg for subsequent experiments. The 5-HT3 receptor antagonist ondansetron showed different effects on intraperitoneal morphine-induced analgesia in normal and SNL rats (fig. 2). Intrathecal pretreatment with ondansetron (3.0 μg) attenuated the analgesic effect of morphine in the normal group (n = 6 in each group, $P = 0.008$; fig. 2, A and C). In contrast, ondansetron (3.0 μg) increased the analgesic effect of morphine in the SNL group (n = 6 in each group, $P = 0.025$; fig. 2, B and C). Administration of ondansetron alone did not affect the withdrawal threshold when compared with saline administration in normal (n = 6 in each group, $P = 0.572$) and SNL rats (n = 6 in each group, $P = 0.747$). Similarly, ablation of serotonergic terminals by intrathecal administration of 5,7-DHT, a selective neurotoxin, produced different effects on intraperitoneal morphine-induced analgesia in normal and SNL rats (fig. 3). Intrathecal administration of 5,7-DHT attenuated the analgesic effect of morphine in normal rats (n = 6 in each group, $P < 0.001$; fig. 3, A and C) but increased the analgesic effect of morphine in SNL rats (n = 6 in each group, $P = 0.001$; fig. 3, B and C). Administration of 5,7-DHT alone did not affect the withdrawal threshold in both normal and SNL rats (the withdrawal thresholds before and 10 days after 5,7-DHT administration were 147.1 ± 10.3 g and 153.3 ± 11.1 g in normal rats and 97.1 ± 9.9 g and 94.6 ± 8.9 g in SNL rats, $P = 0.063$ and $P = 0.526$ by paired *t* test, respectively, n = 12 in each group).

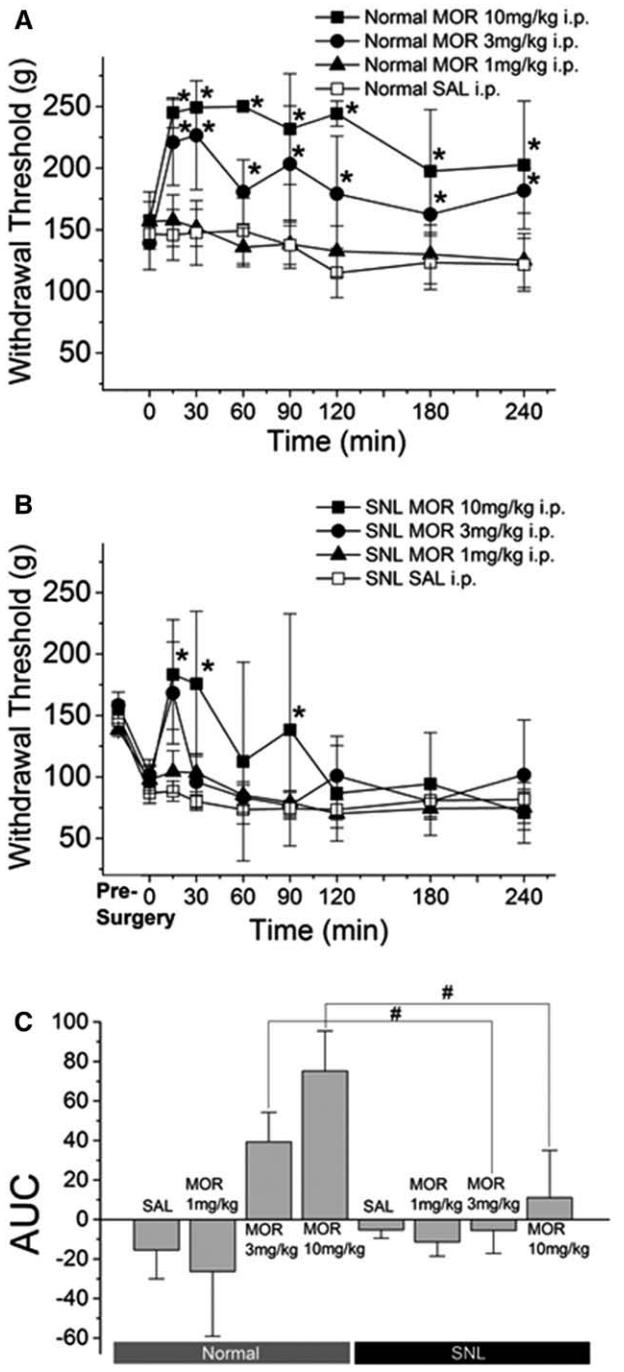

Fig. 1. Effect of intraperitoneal (i.p.) administration of morphine (MOR) or saline (SAL) on withdrawal threshold to pressure applied to the hind paw in normal (*A*) and spinal nerve ligation (SNL) rats (*B*). Time-course effects (*A*, *B*) and area under the time-course curve (AUC) (*C*) are shown. Withdrawal thresholds are expressed as the mean ± SD for six rats in each group. *$P < 0.05$ compared with saline-treated rats at each time point (two-way repeated-measures ANOVA followed by Student–Newman–Keuls *post hoc* tests). #$P < 0.05$ compared between the groups (one-way ANOVA followed by Student–Newman–Keuls *post hoc* tests).

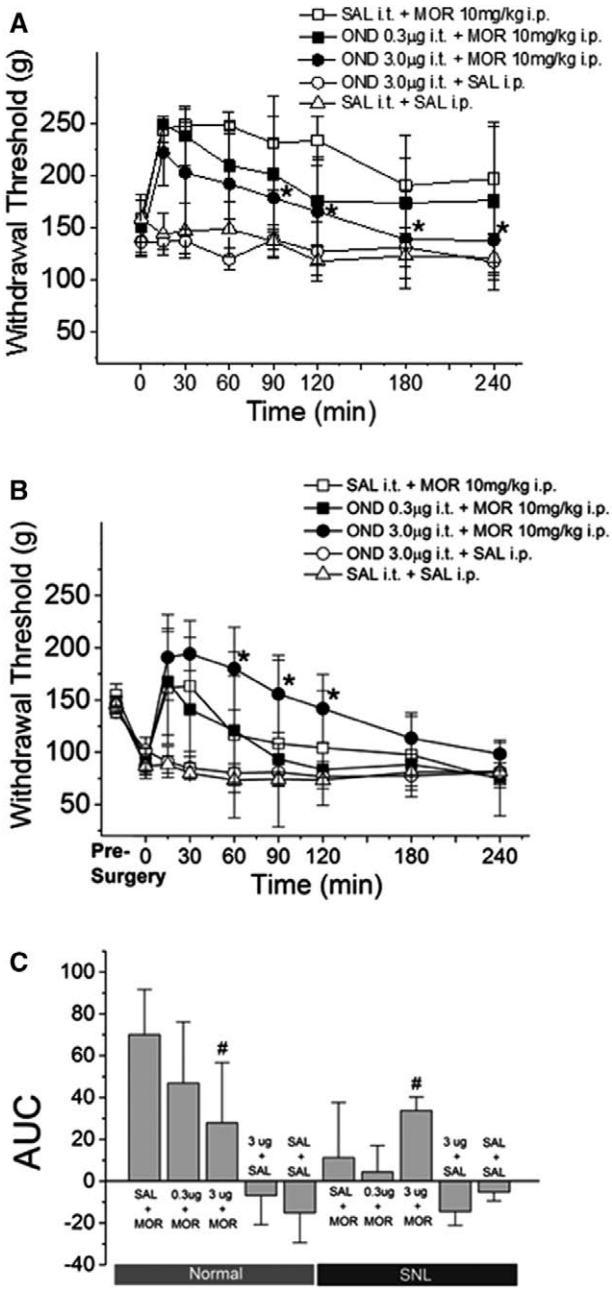

Fig. 2. Effects of intrathecal (i.t.) pretreatment of ondansetron (OND), a 5-hydroxytryptamine 3 receptor antagonist (0.3 or 3 μg), on intraperitoneal (i.p.) morphine (MOR)-induced analgesia (10 mg/kg) in normal (*A*) and spinal nerve ligation (SNL) rats (*B*). Time-course effects (*A*, *B*) and area under the time-course curve (AUC) (*C*) are shown. Withdrawal thresholds are expressed as the mean ± SD for six rats in each group. *$P < 0.05$ compared with the saline (SAL) plus morphine-treated group at each time point (two-way repeated-measures ANOVA followed by Student–Newman–Keuls *post hoc* tests). #$P < 0.05$ compared with the saline plus morphine-treated group (one-way ANOVA followed by Student–Newman–Keuls *post hoc* tests).

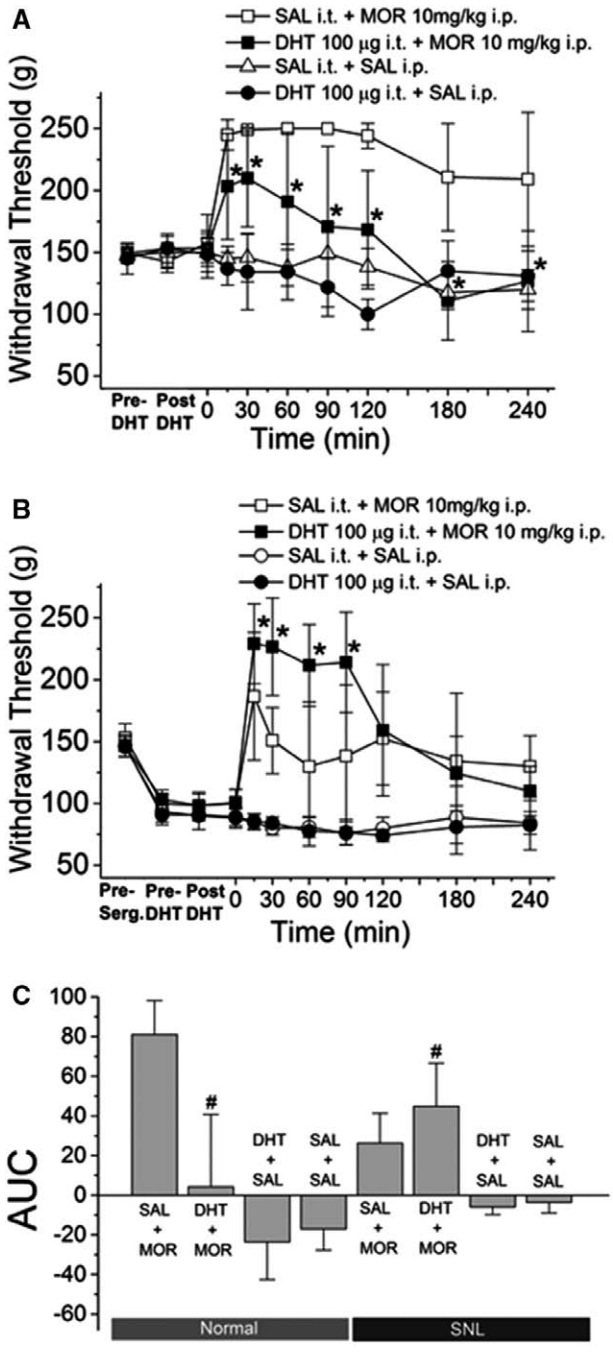

Fig. 3. Effects of intrathecal (i.t.) pretreatment of 5,7-dihydroxytryptamine (DHT) creatinine sulfate (100 μg), a selective neurotoxin for serotonergic terminals, on intraperitoneal (i.p.) morphine (MOR)-induced analgesia (10 mg/kg) in normal (*A*) and spinal nerve ligation (SNL) rats (*B*). Time-course effects (*A*, *B*) and area under the time-course curve (AUC) (*C*) are shown. Withdrawal thresholds are expressed as the mean ± SD for six rats in each group. **P* < 0.05 compared with saline (SAL) plus morphine-treated group at each time point (two-way repeated-measures ANOVA followed by Student–Newman–Keuls *post hoc* tests). #*P* < 0.05 compared with the saline plus morphine-treated group (one-way ANOVA followed by Student–Newman–Keuls *post hoc* tests).

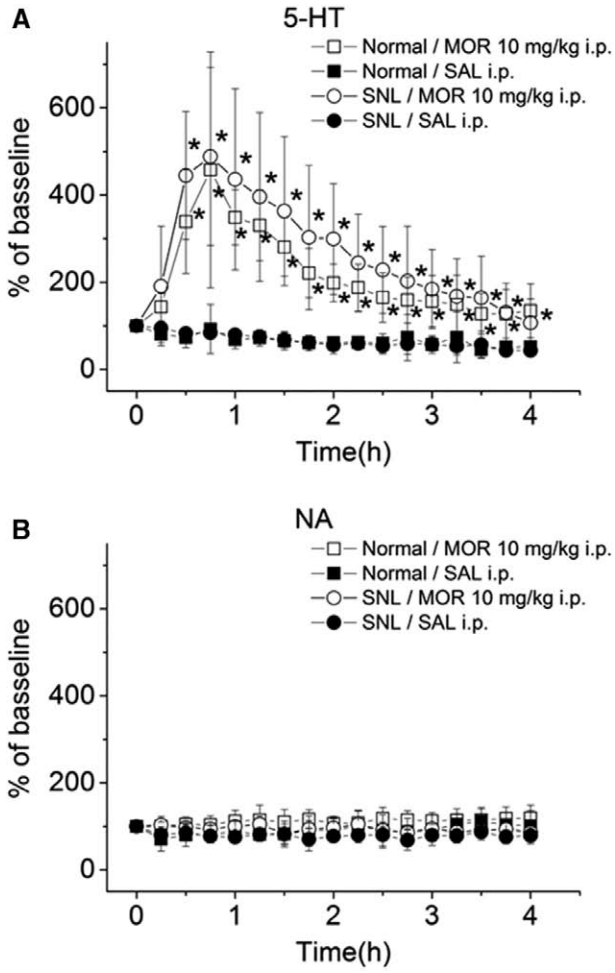

Fig. 4. Microdialysis from the dorsal horn of the lumbar spinal cord to measure levels of 5-hydroxytryptamine (5-HT) (*A*) and noradrenaline (NA) (*B*) after morphine (MOR) injection. Normal rats (n = 6) or spinal nerve ligation (SNL) rats (n = 6) received intraperitoneal (i.p.) saline (SAL) or morphine (10 mg/kg). Data are presented over time as a percentage of the baseline. **P* < 0.05 compared with the saline-treated group at each time point (two-way repeated-measures ANOVA followed by Student–Newman–Keuls *post hoc* tests).

Microdialysis Studies

The baseline 5-HT concentration in spinal cord dorsal horn microdialysates before drug injection did not differ between normal rats (n = 6, 0.36 ± 0.22 pg/15 μl) and SNL rats (n = 6, 0.23 ± 0.13 pg/15 μl). The baseline noradrenaline concentration before drug injection was also similar in both groups (n = 6 in each group, 0.41 ± 0.30 pg/15 μl, normal; 0.45 ± 0.23 pg/15 μl, SNL). The 5-HT and noradrenaline concentrations in the dialysates did not change after saline injection in normal and SNL rats (fig. 4, A and B). In the morphine-treated normal and SNL rats, the 5-HT concentration in the dialysates increased within 30 min, peaked at 1 h with approximately 450 to 500% of the baseline value, and remained increased for 4 h after injection relative to the saline-treated controls (n = 6 in each group,

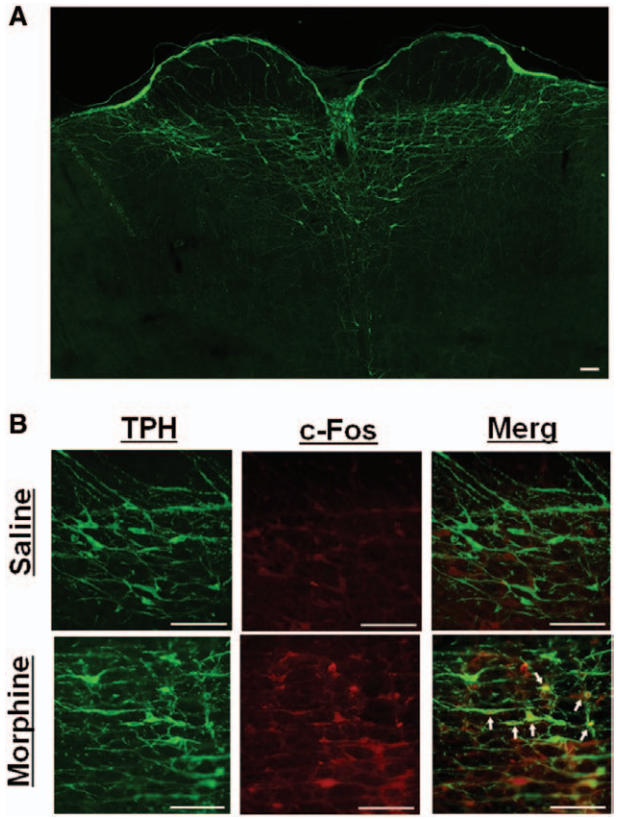

Fig. 5. The rostral ventromedial medulla has been defined as an isosceles triangle, the base of which is the width of the pyramidal tracts (*A*). Images showing tryptophan hydroxylase (TPH) immunoreactivity and c-Fos immunoreactivity in the rostral ventromedial medulla of spinal nerve ligation rats treated with saline and morphine (*B*). *Arrows* indicate serotonergic neurons that would be counted as c-Fos–positive neurons. *Scale bar* = 100 μm.

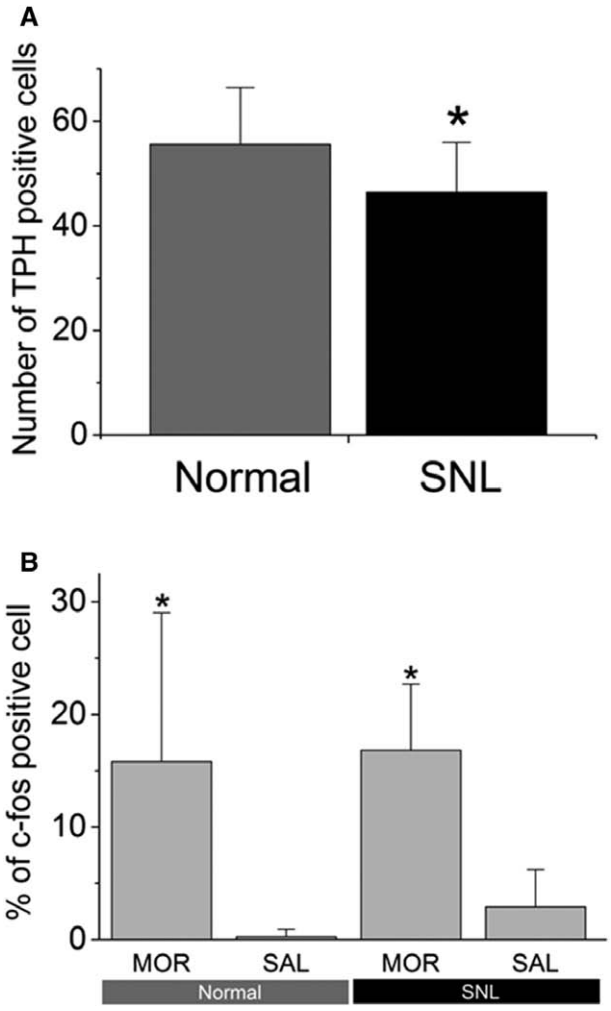

Fig. 6. Spinal nerve ligation (SNL)-induced loss of tryptophan hydroxylase (TPH)-positive neurons in the rostral ventromedial medulla (*A*). Data are expressed as the mean ± SD for 12 rats in each group. *$P < 0.05$ compared with normal rats (one-way ANOVA). Systemic administration of morphine (MOR, 10 mg/kg) increased the percentage of c-Fos–positive serotonergic neurons compared with saline (SAL) treatment in both normal and SNL rats (*B*). Data are expressed as the mean ± SD for six rats in each group. *$P < 0.05$ compared with saline-treated rats (one-way ANOVA).

$P < 0.001$, respectively; fig. 4A). The morphine-induced 5-HT increase did not differ between normal and SNL rats. In the morphine-treated normal and SNL rats, the noradrenaline concentration in the dialysates did not change over time (n = 6 in each group, $P = 0.134$ and $P = 0.305$, respectively; fig. 4B).

Immunohistochemical Studies

The RVM has been defined anatomically as an isosceles triangle that lies at the level of the facial nucleus, with a base having a width equal to that of the combined pyramidal tracts and a height equal to half the width of the base (fig. 5A).[26] c-Fos–positive serotonergic neurons were observed after morphine administration (fig. 5B). SNL resulted in a significant decrease in the number of serotonergic neurons in the RVM (55.6 ± 10.9 *vs.* 46.4 ± 9.5 for normal and SNL, respectively, n = 12 in each group; fig. 6A; $P = 0.039$). Systemic administration of morphine increased the percentage of c-Fos–positive serotonergic neurons compared with administration of saline in both normal rats (n = 6 in each group, 15.8 ± 13.2% *vs.* 0.3 ± 0.7%, $P = 0.016$) and SNL rats (n = 6 in each group, 16.8 ± 5.9% *vs.* 2.9 ± 3.3%, $P < 0.01$; fig. 6B). There were no significant differences in the percentage of c-Fos–positive serotonergic neurons between normal and SNL rats.

Discussion

Clinical studies have reported relatively poor efficacy for morphine in neuropathic pain compared with nociceptive pain,[17–19] and opioids are not used as first-line medications for the management of neuropathic pain.[27] Similarly, laboratory studies have reported a lack of efficacy for intrathecal administration of morphine in a neuropathic pain model in rats,[28,29] although such findings are controversial.[30,31] Few laboratory studies, however, have directly compared the

analgesic effect of morphine in the normal state with that of morphine in neuropathic pain. Here, we demonstrated that the efficacy of systemically administered morphine against mechanical hyperalgesia after nerve injury was reduced compared with its acute antinociceptive effect. To our knowledge, this is the first study to reveal a relative lack of analgesia upon systemic administration of morphine in an animal model of neuropathic pain when compared with the normal state. In the current study, we also obtained other new findings. First, serotonergic descending inhibition of pain transmission was involved in morphine-induced analgesia under the normal state. However, the noradrenergic system was not activated by systemic morphine. Second, the character of serotonergic descending modulation changed from inhibitory to facilitatory after nerve injury through 5-HT3 receptors in the spinal cord.

The analgesic effects of systemically administered morphine have been shown to be mediated in part by descending bulbospinal projections that inhibit dorsal horn neuronal responses to noxious stimuli.[1,7–9] μ-Opioid receptors are densely expressed in the PAG and RVM.[32–34] The PAG projects to the RVM,[35] and morphine-induced PAG activation stimulates RVM output neurons synergistically with the direct effect of morphine on μ-opioid receptors in RVM neurons.[36–38] The PAG also projects to locus coeruleus,[39] and these supraspinal effects are then thought to project through descending serotonergic and noradrenergic neurons to the spinal cord.[40] In the current study, the 5-HT concentration in the lumbar spinal cord increased in normal and SNL rats after injection of intraperitoneal morphine when compared with saline treatment, which agrees with previous reports that also suggested that morphine induces spinal 5-HT increase.[7–9] Furthermore, in the current study, the systemic morphine-induced 5-HT increase in the spinal cord coincided with activation of serotonergic neurons in the RVM as determined by immunohistochemistry.

It is widely accepted that descending noradrenergic inhibition also plays a role in analgesia produced by opioids.[1,41,42] Systemic administration of opioids is believed to induce spinal noradrenaline release. However, only three studies have directly tested this hypothesis and the results are controversial. In a study in sheep, intravenous administration of morphine (1 mg/kg) increased noradrenaline levels in the cerebrospinal fluid and spinal dorsal horn.[43] However, in two recent studies in rats, intraperitoneal administration of morphine (10 mg/kg) reduced noradrenaline levels in the cerebrospinal fluid.[44,45] In the current study, the noradrenaline concentration in the lumbar spinal cord did not change, even though we previously used the same microdialysis technique to detect a three- to five-fold increase in noradrenaline levels after systemic administration of noradrenaline reuptake inhibitors, including antidepressants.[24,25] Therefore, our study suggests that spinal noradrenaline release actually plays a minimal role, if any, in systemic morphine-induced analgesia.

The RVM is recognized as a critical relay site for integrating descending modulatory inputs to the spinal cord.[3,4] Serotonergic cell bodies in the raphe magnus nucleus provide dense projections to the dorsal horn of the spinal cord, and this descending pathway has been shown to mediate the antinociceptive action of systemic morphine.[10,11,46] However, it has also been demonstrated that activation of supraspinal facilitatory pathways from the RVM maintains the abnormal, enhanced pain state associated with peripheral nerve injury.[12–15] Experimentally produced neuropathic pain can be reversed by lidocaine microinjection into the RVM, supporting the critical role of descending facilitation in chronic pain.[15,47] Although the function of serotonergic neurons in the RVM remains unclear, they may play both inhibitory and facilitatory roles in nociceptive transmission.[10,11] In SNL, Leong *et al.*[48] demonstrated that the number of RVM neurons, including serotonergic neurons, decreased, and the remaining serotonergic neurons mediated descending facilitation. Leong *et al.*[48] thus proposed that the loss of RVM neurons shifts the balance of descending control from pain inhibition to pain facilitation. In the current study, we also found that SNL significantly decreased the number of serotonergic neurons in the RVM. Therefore, degeneration of inhibitory serotonergic neurons in RVM may underlie the reduced efficacy of systemic morphine for neuropathic pain.

Chronic pain states are associated with enhanced descending facilitation of pain signaling, mediated in part through activation of excitatory spinal 5-HT3 receptors.[4,13,16] 5-HT3 receptors are expressed in the terminals of primary afferents[49] and also in the somadendritic regions and presynaptic terminals of γ-aminobutyric acid neurons in the spinal dorsal horn.[50] Stimulation of 5-HT3 receptors in the spinal cord can thus result in facilitation of pain transmission by increasing neurotransmitter release from the primary sensory afferents[51] or inhibition of pain transmission by increasing γ-aminobutyric acid release.[50,52] Animal studies have suggested that spinal 5-HT3 receptor blockade[4,13] attenuates nerve injury–induced hypersensitivity, and a clinical study demonstrated that ondansetron can attenuate chronic pain.[53] Paradoxically, 5-HT3 receptor agonists also reduce hypersensitivity in some animal pain models.[54,55] Thus, increased 5-HT levels in the spinal cord after systemic morphine administration likely result in activation of both inhibitory and facilitatory pathways through spinal 5-HT3 receptors, and the efficacy of morphine may therefore depend on the balance between these pathways.[56] Many studies have supported the dysfunction of γ-aminobutyric acidergic inhibition in the spinal cord after nerve injury, based on immunohistochemistry[57–60] or electrophysiological recordings.[58,59,61,62] We speculate that these functional changes in γ-aminobutyric acidergic interneurons in the spinal dorsal horn may underlie the switching of spinal 5-HT3 receptor activation from antinociceptive in

the normal state to prohyperalgesic in the setting of neuropathic pain.

The current ondansetron and 5,7-DHT experiments suggest that local activation of spinal 5-HT3 receptors by 5-HT released from spinally projecting RVM neurons upon systemic morphine administration facilitates analgesia in the normal state but blunts analgesia in neuropathic pain. Together with our observation of reduced numbers of putative opioid-activated descending inhibitory neurons in the RVM after SNL, these results support the idea that nerve injury leading to neuropathic pain shifts the balance of serotonergic nociceptive modulation toward descending facilitation of pain transmission and thus decreases the analgesic efficacy of morphine. Our results also suggest that this descending pain facilitation may at least in part be blocked by 5-HT3 antagonism, which is highly relevant because 5-HT3 antagonists, such as ondansetron, are already used clinically as antiemetic agents for patients treated with opioids. Therefore, 5-HT3 antagonists may be a promising tool to enhance the analgesic effects of morphine in neuropathic pain.

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Competing Interests

The authors declare no competing interests.

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Holy Moly: Hermes' Anticholinesterase Antidote

Above the double-flowering sweet violets (ca.1870, *right*), W. H. Prestele depicted Snowdrops from the genus *Galanthus* of winter/spring blooming plants with dark roots and down-facing white flowers. Snowdrops were linked (1722) by British poet Thomas Tickell to the herb Moly that wing-helmeted Hermes (*left*, A. Carracci's ca.1590 *Mercury Protecting Ulysses from the Charms of Circe*) gave Odysseus (Ulysses) to counteract the potion of the witch-goddess Circe. Plaitakis and Duvoisin (1983) have suggested that the Snowdrop's anticholinesterase inhibitor, galant(h)amine, could be apotropaic (warding off evil) against the tropane alkaloidal effects of Circe's anticholinergic potion.

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