Activation of Peripheral μ-opioid Receptors by Dermorphin [D-Arg2, Lys4] (1–4) Amide Leads to Modality-preferred Inhibition of Neuropathic Pain

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ABSTRACT

Background: Opioids have long been regarded as the most effective drugs for the treatment of severe acute and chronic pain. Unfortunately, their therapeutic efficacy and clinical utility have been limited because of central and peripheral side effects.

Methods: To determine the therapeutic value of peripheral μ-opioid receptors as a target for neuropathic pain treatment, the authors examined the effects of dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA), a hydrophilic, peripherally acting μ-opioid receptor agonist, in male and female rats with spinal nerve ligation–induced neuropathic pain. The authors also utilized behavioral, pharmacologic, electrophysiologic, and molecular biologic tools to characterize DALDA's possible mechanisms of action in male rats.

Results: DALDA, administered subcutaneously, had 70 times greater efficacy for inhibiting thermal (n = 8 to 11/group) than mechanical hypersensitivity (n = 6 to 8/group) in male rats. The pain inhibitory effects of DALDA on mechanical and heat hypersensitivity were abolished in animals pretreated with systemic methylnaltrexone (n = 7 to 9/group), a peripheral μ-opioid receptor antagonist. In the spinal wide-dynamic range neurons, systemic DALDA inhibited C-fiber–mediated, but not A-fiber–mediated, response in neuropathic male rats (n = 13). In primary sensory neurons, DALDA inhibited the capsaicin-induced [Ca2+] increase more than the β-alanine–induced [Ca^{2+}] increase (n = 300); capsaicin and β-alanine activate subpopulations of neurons involved in the signaling of heat and mechanical pain, respectively. DALDA-treated rats (n = 5 to 8/group) did not exhibit motor deficits and locomotor impairment suggesting that it does not induce central side effects.

Conclusions: These findings suggest that DALDA may represent a potential alternative to current opioid therapy for the treatment of neuropathic pain and is likely to be associated with minimal adverse effects. **(Anesthesiology 2016; 124:706-20)**

CHRONIC neuropathic pain is prevalent among 6 to 8% of the population. In patients attending pain clinics, the incidence is as high as 25 to 51.9%.[1] Moreover, chronic pain causes considerable social and economic burden and leads to high healthcare costs and lost productivity.[2,3] Patients with neuropathic pain resulting from nerve injuries present with varying degrees of mechanical and heat hyperalgesia. These manifestations are also observed in animal models of neuropathic pain.[4,5] Mechanical and heat hypersensitivities involve different peripheral and central mechanisms and hence may require different treatment strategies.[4,6,7] In the past several years, a number of drugs have been developed for the treatment of neuropathic pain, but no single agent is uniformly effective, and opioids remain some of the most commonly used drugs. However, their therapeutic utility is limited considerably by their severe central and peripheral side effects, including sedation, dizziness,

What We Already Know about This Topic

- A number of drugs have been developed for the treatment of neuropathic pain, but no single agent is uniformly effective, and opioids remain some of the most commonly used drugs. Activating opioid receptors in the peripheral nervous system may offer an opportunity for treating certain chronic pain conditions while avoiding their central side effects.

What This Article Tells Us That Is New

- By using a model of male and female rats with spinal nerve ligation–induced neuropathic pain, the authors demonstrated that systemic administration of dermorphin [D-Arg2, Lys4] (1–4) amide, a highly selective μ-opioid receptor agonist, attenuated both neuropathic mechanical and heat hypersensitivity through activation of μ-opioid receptor at peripheral but not central sites. Further, the efficacy of dermorphin [D-Arg2, Lys4] (1–4) amide to inhibit heat hypersensitivity is greater than that to inhibit mechanical hypersensitivity.

The first three authors (Vinod Tiwari, F.Y., S.-Q.H.) contributed equally to this work. Vinod Tiwari, F.Y., and S.-Q.H. performed most of the experiments and were involved in writing a draft manuscript. R.S., C.Z., B.S., T.Z., and Vineeta Tiwari performed or assisted with portions of the experiments. Y.W. and X.D. were involved in experimental design, data analysis, and interpretation. Y.G. and S.N.R. designed and directed the project and wrote the final manuscript.

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respiratory depression, constipation, nausea, vomiting, tolerance, and physical dependence.[8] These adverse effects may lead to opioid discontinuation and contribute to underdosing and inadequate analgesia in patients with neuropathic pain.[9] The potential adverse effects are a primary reason that opioids have been downgraded from a second-line class to a third-line class of drugs in the recent recommendations for the pharmacologic treatment of neuropathic pain.[10] Hence, alternative therapies that lack central and peripheral adverse effects are important for the treatment of neuropathic pain.

Activating opioid receptors in the peripheral nervous system may offer an opportunity for treating certain chronic pain conditions while avoiding their central side effects.[11,12] However, whether peripheral opioids affect mechanical and heat hypersensitivity differently is unclear. In addition, little is known about their cellular mechanisms, such as which subpopulations of primary sensory neurons are targeted by peripherally acting opioids to inhibit different neuropathic pain modalities. Dermorphin is a natural heptapeptide μ-opioid receptor (MOR) agonist found in amphibian skin.[13,14] Degradation of dermorphin by peptidases produces the *N*-terminal tetrapeptide H-Tyr-D-Ala-Phe-Gly-OH.[14,15] Additional amino acid substitutions to this tetrapeptide led to the development of dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA), a highly selective MOR agonist.[16–18] Intriguingly, DALDA is highly hydrophilic because it carries three net positive charges at physiologic pH. Moreover, it is metabolically more stable than its other analogs and exhibits a restrictive penetration into the central nervous system (CNS) after systemic drug administration.[17] These properties make DALDA a promising drug candidate for the treatment of chronic neuropathic pain with reduced risk of central side effects.

Critical to the use of peripherally acting opioids is an understanding of the analgesic properties and mechanisms that underlie the therapeutic effects of DALDA. Therefore, the purpose of this research was to improve our understanding of the cellular mechanisms involved in the peripheral opioid analgesia and the development of peripheral opioids with minimal central side effects. In this study, we characterized the efficacy of systemic DALDA to attenuate mechanical and heat hypersensitivity in nerve-injured rats, investigated its site and mechanism of action, and assessed its safety profile.

Materials and Methods

Animals

Male and female Sprague-Dawley rats (200 to 350 g; Harlan Bioproducts for Science, USA) were housed under optimal laboratory conditions with a 12-h light/dark cycle and free access to food and water. Animals were acclimatized to laboratory conditions before the tests. All behavioral experiments were carried out between 9:00 AM and 5:00 PM by an investigator blinded to the drug assignment. The experimental protocols were approved by the Animal Care and Use Committee of Johns Hopkins University and complied with the National Institutes of Health Guide for the Use of Experimental Animals to ensure minimal animal use and discomfort.

Drugs

DALDA was purchased from US Biologicals (USA), and methylnaltrexone bromide and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP) were purchased from Sigma-Aldrich (USA). Other drugs were purchased from Sigma-Aldrich or Tocris Bioscience (United Kingdom). Stock solutions were freshly prepared as instructed by the manufacturer.

Neuropathic Pain Model

L5 spinal nerve ligation (SNL) surgery was used for the induction of neuropathic pain in rats. The procedure was a modification of that described in our previous studies.[19,20]

Intrathecal Catheter Implantation

After rats were anesthetized (2% isoflurane), a small slit was cut in the atlantooccipital membrane, and a 6- to 7-cm piece of saline-filled PE-10 tubing was inserted. We confirmed intrathecal drug delivery by injecting lidocaine (400 μg/20 μl, Hospira, USA), which resulted in a temporary motor paralysis of the lower limbs.[19,20]

Animal Behavioral Tests

Animals were allowed to recover from surgery for 2 weeks before any behavior testing was done. Animals were acclimatized and habituated to the test environment. All procedures have been described in our previous studies.[19–21]

von Frey Hair Test. To assess mechanical hypersensitivity to punctuate mechanical stimuli, we measured paw withdrawal threshold (PWT) to von Frey filaments. Each filament (0.38 to 13.1 g) was applied to the test area on the plantar surface of the hind paw for 4 to 6 s according to the up-down method.[21–23]

Hargreaves Test. To test for signs of heat hypersensitivity, we used the Hargreaves test, which measures paw withdrawal latency (PWL) to radiant heat stimuli. Radiant heat was applied to the plantar surface of each hind paw for three times (3- to 5-min interval) with a plantar stimulator analgesia meter (IITC model 390, USA). A cutoff time of 20 s was used to prevent tissue damage.

Rota-rod Test. We used the rota-rod test to assess the well-known motor impairment side effects of opioids. Rats were acclimatized and trained on a rotating rod (Ugo Basile, Italy) that accelerated from 0 to 30 rpm in 180 s. On the day of testing, rat performance on the rod was measured before (predrug baseline) and 45 min after administration of DALDA or morphine. The time (in seconds) that each animal remained on the accelerating rod without falling was recorded.[19,24]

Open Field Test. The open field test was used to assess the effect of systemic DALDA administration on spontaneous exploration and locomotor activity of rats. Rats were placed in an open field chamber (73 × 45-cm rectangular plastic box with a wall height of 33 cm) for 10 min. Their behavior was video recorded, and parameters such as total distance travelled; mean travel speed; and number of border periphery, internal periphery, and center crossings were analyzed by SMART 3 software (Panlab Harvard Apparatus, USA).

Spinal Dorsal Horn Recordings

In anesthetized rats, we performed tracheotomy, mechanical ventilation, and extracellular recordings of single dorsal horn neuronal activity as described in our previous studies.[20,21] Briefly, a laminectomy was performed at vertebral levels T12 to L1 corresponding to lumbar enlargements at spinal segments L3 to S1. During neurophysiologic recording, animals were paralyzed with intraperitoneal pancuronium bromide (0.15 mg/kg, Elkins-Sinn Inc., USA) to facilitate controlled ventilation. Only wide-dynamic range (WDR) neurons with defined receptive fields (RFs) in the plantar region of the hind paw were studied. The cutaneous RFs of WDR neurons were mapped, and a single site (most sensitive site) near the center of the RF was chosen for application of test stimuli. Analog data were collected with a real-time, computer-based data acquisition and processing system (CED Spike 2, United Kingdom). WDR cells were identified by their characteristic responses.[20,21] The evoked responses of WDR neurons to a series of mechanical (brushing, graded von Frey monofilaments: 1 to 15 g, Stoelting Co., USA) and electrical test stimuli (0.1 to 10 mA, 2 ms) were examined. The WDR neuronal response to a suprathreshold electrical stimulus consists of an early A-component (0 to 100 ms) and a later C-component (100 to 500 ms).

Dorsal Root Ganglion Neuronal Culture and Calcium Imaging

Experiments were conducted as we have described previously.[19,25,26] Briefly, dorsal root ganglions (DRGs) from rats were collected in cold DH10 medium and treated with enzyme solution at 37°C. Neurons were loaded with Fura-2-acetomethoxyl ester (Molecular Probes, USA) for 45 min in the dark at room temperature.[25,26] After being washed, cells were imaged at 340 and 380 nm excitation for detection of intracellular free calcium. Calcium imaging assays were performed by an experimenter blind to drug treatment. For isolectin IB4 labeling studies, dissociated DRG neurons were cultured in an incubator at 37°C. After 24 to 48 h, neuron cultures were treated with fluorescein-labeled *Griffonia simplicifolia* lectin I-isolectin B4 (1:500; Vector Laboratories, USA) for 10 min at room temperature and rinsed for 3 min. Then these cultures were loaded with Fura-2-acetomethoxyl ester. After calcium imaging assays, we selected small-diameter DRG neurons (less than 25 μm) to analyze the changes in calcium concentration ($[Ca^{2+}]$). Cells were divided into two groups: $IB4^-$ and $IB4^+$ neurons.

Statistical Analysis

To establish the dose–response functions, we normalized PWT and PWL data by calculating maximum possible effect (MPE) values, as PWTs are often at the cutoff values in naive animals. MPE values for inhibiting mechanical hypersensitivity were calculated with the equation: MPE (%) = [1 – (Cutoff PWT – Postdrug PWT)/(Cutoff PWT – Predrug PWT)] × 100, where cutoff PWT = 21.5 g. Because PWLs in naïve animals do not reach the cutoff value (20 s), MPE values for inhibiting heat hypersensitivity were calculated with the equation: MPE (%) = [1 – (Preinjury PWL – Postdrug PWL)/(Preinjury PWL – Predrug PWL)] × 100. Thus, the MPE value for inhibiting heat hyperalgesia may exceed 100%. In electrophysiology studies, we compared the number of action potentials evoked by test stimuli between predrug and postdrug conditions. For analysis of windup, we plotted the C-component of WDR neurons evoked by each stimulus against the stimulation number in a train of 16 stimuli. We compared the A- and C-component produced by graded electrical stimuli and total C-component in response to windup stimulation between predrug and postdrug conditions.

There were no data missing for any of the variables. The methods for statistical comparisons in each study are given in the figures. The number of animals used in each study was based on our experience with similar studies. We randomized animals to the different treatment groups and blinded the experimenter to drug treatment to reduce selection and observation bias. STATISTICA 6.0 software (StatSoft, Inc., USA) was used to conduct all statistical analyses. The Tukey honestly significant difference *post hoc* test was used to compare specific data points. Bonferroni correction was applied for multiple comparisons. Two-tailed tests were performed; $P < 0.05$ was considered significant in all tests.

Results

DALDA-induced Attenuation of Mechanical Hypersensitivity Involves Activation of Peripheral μ-opioid Receptors

To investigate the therapeutic utility of DALDA on neuropathic pain, we first examined the effects of systemic administration of DALDA on SNL-induced mechanical hypersensitivity. Compared with preinjury baseline, paw withdrawal threshold (PWT) of nerve-injured (ipsilateral) hind paw to mechanical stimuli was significantly decreased at 2 to 3 weeks post-SNL. Subcutaneous administration of DALDA in male SNL rats dose-dependently increased the ipsilateral PWTs at 15, 45, and 120 min (0.2 to 10 mg/kg, n = 6 to 8/dose), compared with the predrug baseline (fig. 1A). The magnitude and duration of DALDA-induced antiallodynic effects increased with dose. We calculated the peak MPE at 45 min postdrug to establish the dose–response function and calculated the dose estimated to produce 50% MPE (ED_{50}) as 4.2 mg/kg. MPEs of 5 and 10 mg/kg doses were

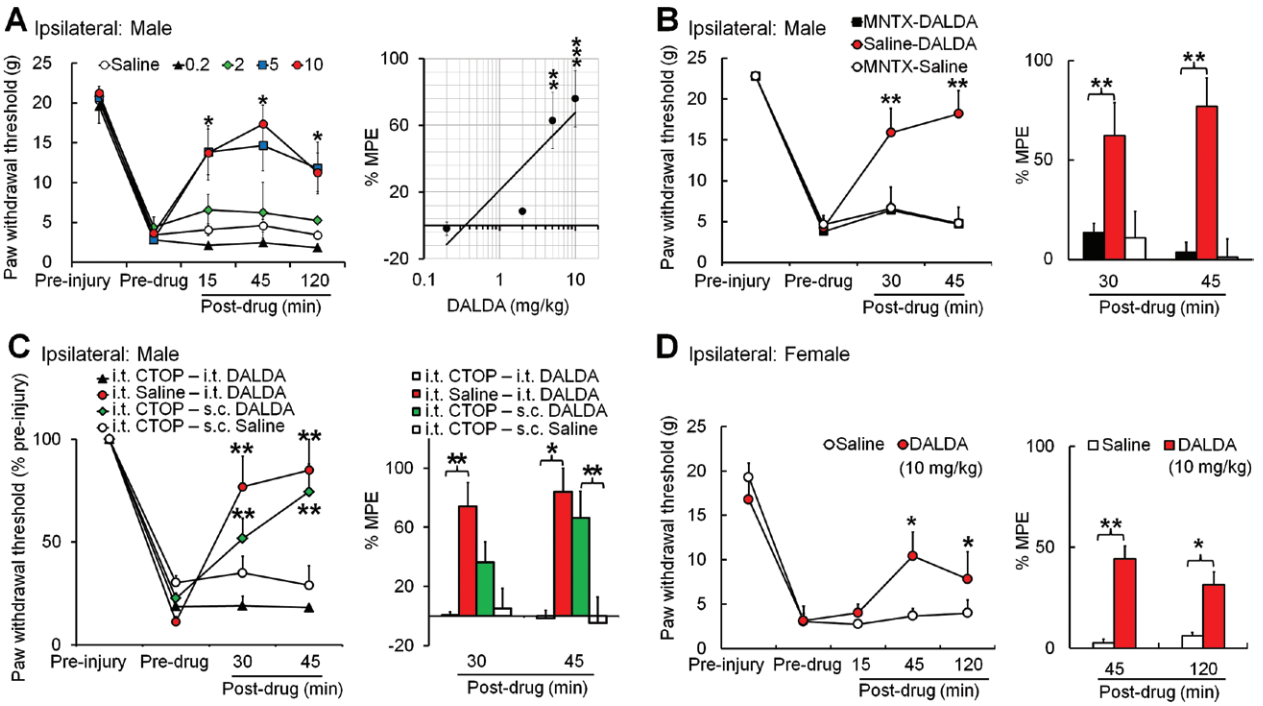

Fig. 1. Systemic administration of dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA) inhibits mechanical hypersensitivity in nerve-injured male rats through activation of peripheral opioid receptors. (*A*, *left*) Subcutaneous (s.c.) injection of DALDA (0.2, 2, 5, and 10 mg/kg, n = 6 to 8/dose) dose-dependently inhibited mechanical allodynia in male rats at 2 to 3 weeks after spinal nerve ligation (SNL), as indicated by the significant increase in ipsilateral paw withdrawal threshold (PWT). The time course shows peak drug effect at 45 min after injection. **P* < 0.05 *versus* predrug (5 and 10 mg/kg), two-way mixed model ANOVA. (*A, right*) The maximum possible effect (MPE) of DALDA at 45 min postdrug was calculated. MPE (%) = [1 – (Preinjury PWT – Postdrug PWT)]/[Preinjury PWT – Predrug PWT] × 100. ***P* < 0.01, ****P* < 0.001 *versus* saline group (n = 11), one-way ANOVA. (*B*, *left*) Pretreatment with an intraperitoneal (i.p.) injection of methylnaltrexone bromide (MNTX, 5 mg/kg, 10-min pretreatment, n = 7), but not saline (n = 7), blocked the inhibitory effect of DALDA (10 mg/kg, s.c.) on mechanical hypersensitivity in male SNL rats. Injection of MNTX (5 mg/kg, i.p., n = 7) followed by saline (s.c.) did not change PWT from predrug baseline. (*B, right*) The MPEs at 30 and 45 min after the second drug administration were calculated for each group. (*C*, *left*) Intrathecal (i.t.) pretreatment with the highly selective MOR antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP, 5 μg/10 μl, n = 5), but not saline (n = 5), blocked inhibition of mechanical hypersensitivity by i.t. DALDA (0.5 μg/10 μl) in male SNL rats. However, the same CTOP treatment did not block inhibition of mechanical hypersensitivity by systemic DALDA (10 mg/kg, s.c., n = 7). Intrathecal CTOP (5 μg/10 μl, n = 5) followed by saline injection (s.c.) did not change PWT from predrug baseline. (*D*, *left*) DALDA (10 mg/kg, n = 6, s.c.), but not saline (n = 6), also inhibited mechanical allodynia in female rats at 2 to 3 weeks after SNL. (*D, right*) The MPE at 45 and 120 min postdrug was calculated. (*B–D*, *left*) **P* < 0.05, ***P* < 0.01 *versus* predrug, two-way mixed model ANOVA. (*B-D, right*) **P* < 0.05, ***P* < 0.01 *versus* saline, one-way ANOVA. Data are expressed as mean ± SEM.

significantly higher than the MPE of vehicle (saline, n = 11, fig. 1A). Contralateral PWT did not change significantly after nerve injury or drug treatment (data not shown). Next, to identify the site of action of DALDA, we utilized peripheral and central MOR antagonists. We pretreated nerve-injured male rats with systemic methylnaltrexone (5 mg/kg, intraperitoneally), a peripherally acting MOR-preferring antagonist, 10 min before DALDA treatment (10 mg/kg, subcutaneously, n = 7). Methylnaltrexone, but not saline, completely blocked the antiallodynic effects of systemic DALDA on mechanical hypersensitivity in male SNL rats (fig. 1B, n = 7). In contrast, pretreatment with intrathecal CTOP (0.5 μg/10 μl, n = 7), a highly selective MOR antagonist, did not prevent the antiallodynic effects of systemic DALDA (fig. 1C). The same CTOP pretreatment blocked spinal opioid analgesia induced by intrathecal injection of DALDA (0.5 μg/10 μl, n = 5), suggesting that CTOP effectively blocks MOR activation in the spinal cord. DALDA (10 mg/kg, subcutaneously) also significantly increased the ipsilateral PWTs from predrug baseline in female rats at 2 to 3 weeks post-SNL (n = 6, fig. 1D). There was a trend that peak MPE at 45 min after DALDA treatment in female rats (44.2 ± 13.3%,10 mg/kg, subcutaneously) is lower than that in male rats (75.9 ± 13.8%, n = 7), but the difference did not reach statistical significance (*P* = 0.11, Student's *t* test).

Less DALDA Is Required to Attenuate Thermal Hyperalgesia than to Inhibit Mechanical Allodynia

To further examine the effect of systemic DALDA on heat hyperalgesia in nerve-injured rats, we measured PWL with the Hargreaves test before and after drug treatment. At 2 to 3 weeks post-SNL, PWL was significantly decreased in the

ipsilateral hind paw (fig. 2A) but not in the contralateral hind paw (fig. 2B). Systemic DALDA dose-dependently attenuated this SNL-induced thermal hyperalgesia in male rats (0.02, 0.1, 0.2, and 2 mg/kg, subcutaneously, n = 8 to 11/group). We used the peak MPEs for systemic DALDA to reverse heat hyperalgesia at 45 min postdrug to establish the dose–response function and calculate ED_{50} (fig. 2C). The ED_{50} (0.06 mg/kg) was significantly lower than that required to inhibit mechanical allodynia (4.2 mg/kg) in male SNL rats. MPEs for 0.1, 0.2, and 2 mg/kg doses were all significantly higher than MPE for the vehicle-treated group (saline, n = 11; fig. 2C). An intraperitoneal injection of methylnaltrexone (5 mg/kg), but not saline (n = 9/group), 10 min before DALDA injection completely blocked the antihyperalgesic effect of high-dose DALDA (2 mg/kg, subcutaneously; fig. 2D). Drug treatment did not significantly alter PWL of the contralateral hind paw (fig. 2E). These findings suggest that peripheral MOR activation mediates systemic DALDA-induced inhibition of both mechanical and heat hyperalgesia in nerve-injured male rats. However, inhibition of mechanical hypersensitivity requires a 70-fold greater dose of DALDA than inhibition of heat hyperalgesia. DALDA (10 mg/kg, subcutaneously) also increased the ipsilateral PWLs from predrug baseline in female rats (n = 6, fig. 2F) but did not significantly alter PWL of the contralateral hind paw (fig. 2G).

Systemic DALDA Inhibits the C-component of Spinal WDR Neurons More Potently than the A-component

To further confirm the site of action and delineate the cellular mechanisms involved in DALDA's modality-preferred inhibition of neuropathic pain, we examined the changes in spinal WDR neuronal response before and after systemic DALDA treatment in male SNL rats. WDR neurons receive converging afferent inputs in both low-threshold A-fibers and high-threshold C-fibers (presumably nociceptive). Yet, the A- and C-fiber–mediated responses to natural stimulation are not readily differentiated in WDR neurons. In contrast, WDR neuronal response to a suprathreshold electrical

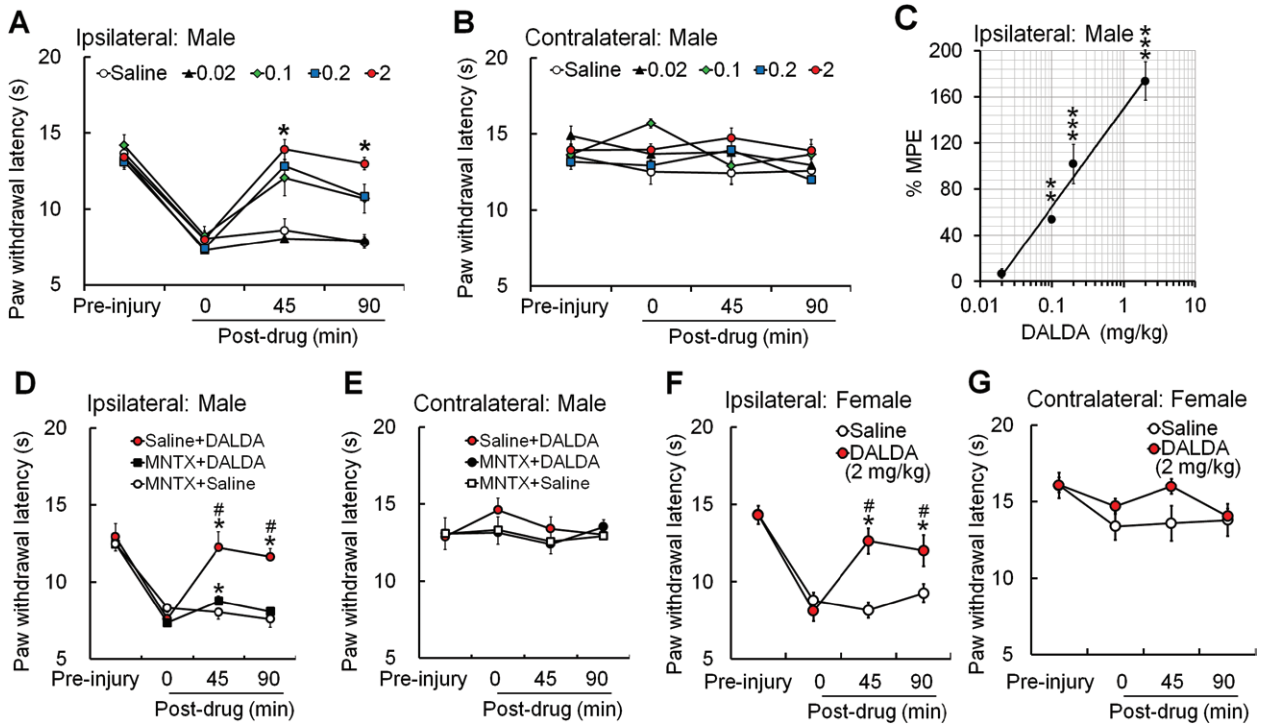

Fig. 2. Systemic administration of dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA) inhibits heat hypersensitivity in nerve-injured rats. (*A*) At 2 to 3 weeks after spinal nerve ligation (SNL) in male rats, subcutaneous injection of DALDA (0.02, 0.1, 0.2, and 2 mg/kg, n = 8 to 11/group) dose-dependently increased the ipsilateral paw withdrawal latency (PWL) compared with that at predrug baseline. **P* < 0.05 *versus* predrug (0.1. 0.2, and 2 mg/kg), two-way mixed model ANOVA. (*B*) The PWL in the contralateral hind paw did not change after nerve injury or drug treatment. (*C*) The maximum possible effect (MPE) of DALDA at 45 min postdrug was calculated. MPE (%) = [1 – (Preinjury PWL – Postdrug PWL)/(Preinjury PWL – Predrug PWL)] × 100. ***P* < 0.01, ****P* < 0.001 *versus* saline group (n = 11), one-way ANOVA. (*D*) Pretreatment with an intraperitoneal injection of methylnaltrexone bromide (MNTX; 5 mg/kg, n = 9), but not saline (n = 9), 10 min before DALDA (2 mg/kg, subcutaneously) blocked inhibition of heat hyperalgesia by DALDA in male rats. MNTX (5 mg/kg) followed by saline (subcutaneously) did not change PWL from predrug baseline (n = 9). **P* < 0.05 *versus* predrug, and #*P* < 0.05 *versus* MNTX + DALDA group, two-way mixed model ANOVA. (*E*) The PWL of the contralateral hind paw did not change after nerve injury or drug treatment. (*F, G*) DALDA (10 mg/kg, n = 6, subcutaneously), but not saline (n = 6), also inhibited heat hypersensitivity in female rats at 2 to 3 weeks after SNL. **P* < 0.05 *versus* predrug, and #*P* < 0.05 *versus* saline, two-way mixed model ANOVA. Data are expressed as mean ± SEM.

stimulus (2 ms) can be separated as a short latency A-fiber component (0 to 100 ms) and a longer latency C-fiber component (100 to 500 ms; fig. 3A), based on the calculated conduction velocities. This unique feature of WDR neuronal response allows us to differentiate the drug effects on A- and C-fiber–mediated activities in the same neuron. We then characterized the effects of DALDA on responses of WDR cells to graded intracutaneous electrical stimuli (0.1 to 10 mA, 2 ms) and to windup-inducing stimuli (16 pulses, 0.5 Hz, supra-C-fiber activation threshold, 2 ms) in nerve-injured rats. An electrical search stimulus was applied through a pair of fine needles inserted subcutaneously at the central area of the hind paw. Systemic DALDA (10 mg/kg, intraperitoneally) significantly decreased the C-component,

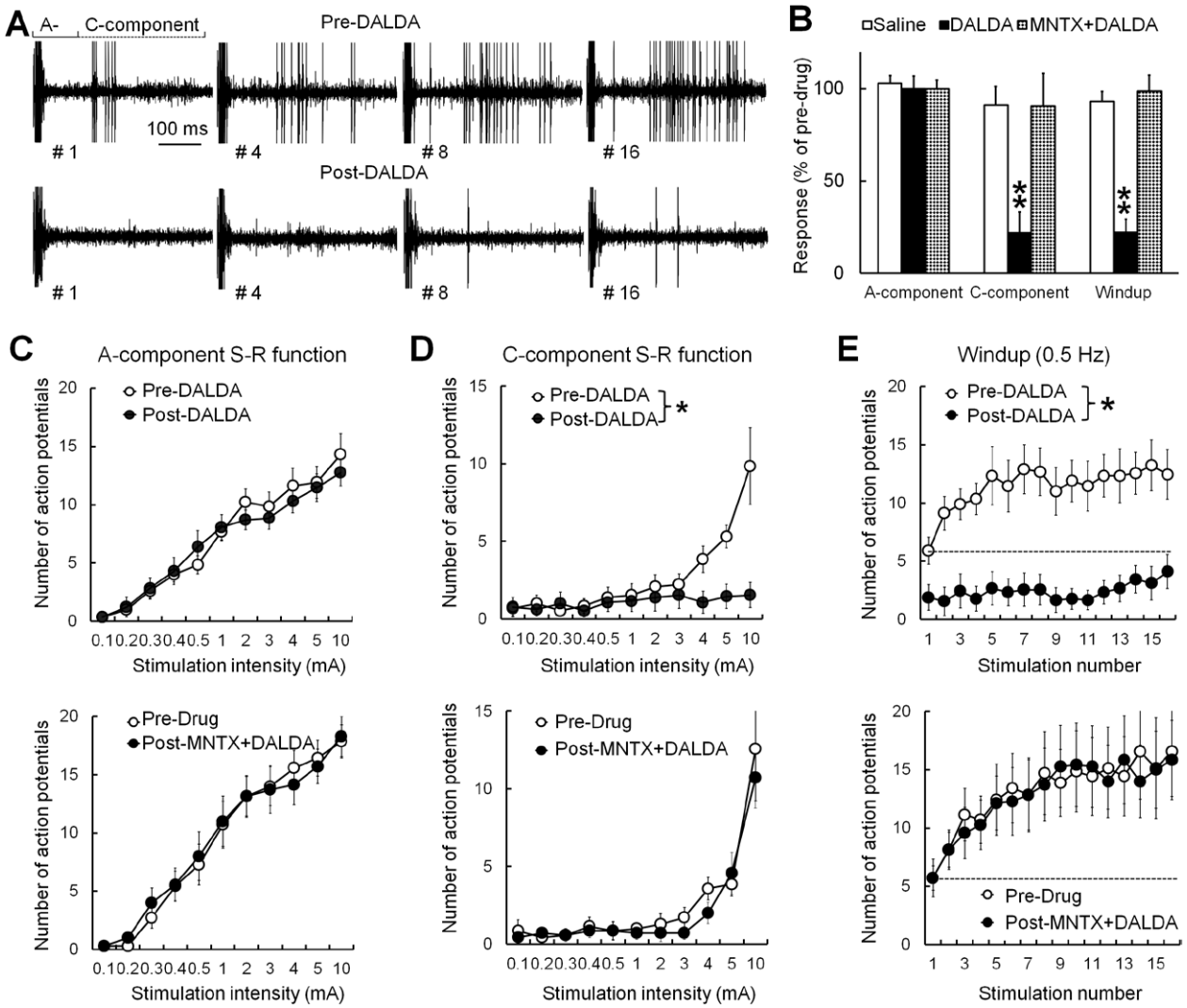

Fig. 3. Systemic dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA) selectively inhibits the C-component of wide-dynamic range (WDR) neurons to electrical stimulation, an effect that is blocked by methylnaltrexone bromide (MNTX). (*A*) An analog recording of WDR neuronal responses to the 1st, 4th, 8th, and 16th stimulus of a train of intracutaneous electrical stimuli (0.5 Hz, 16 pulses, 2.0 ms, supra-C-fiber activation threshold) that induces windup. WDR neuronal responses display A- and C-components to an intracutaneous electrical stimulus. At 2 to 3 weeks after spinal nerve ligation (SNL) in male rats, windup of C-component was inhibited at 30 to 45 min after systemic administration of DALDA (10 mg/kg, intraperitoneally). (*B*) The total A-component and C-component to graded intracutaneous electrical stimuli (0.1 to 10 mA, 2.0 ms) and the total C-component to windup-inducing stimuli after treatment of SNL rats with saline (intraperitoneally, n = 10), DALDA (10 mg/kg, intraperitoneally, n = 13), and MNTX (5 mg/kg, intraperitoneally, 15-min pretreatment, n = 9) followed by DALDA (10 mg/kg). $^{**}P < 0.01$ *versus* saline group, one-way ANOVA. (*C*) The stimulus-response (S-R) function of the A-component of WDR neuronal response to graded intracutaneous electrical stimuli (0.1 to 10 mA, 2 ms) before and 30 to 45 min after systemic injection of DALDA (*upper*, 10 mg/kg, n = 9, intraperitoneally) or MNTX (5 mg/kg, intraperitoneally, 15-min pretreatment) with DALDA (*lower*, 10 mg/kg, n = 8, intraperitoneally). (*D*) The S-R function of C-component to graded intracutaneous electrical stimuli in each group. (*E*) Windup of C-component of WDR neurons to a train of intracutaneous electrical stimuli (0.5 Hz, 16 pulses) before and after drug treatment. The C-component to 0.5 Hz stimulation was plotted against the stimulation sequence number of each trial. (*C–E*) $^{*}P < 0.05$ *versus* predrug, two-way repeated measures ANOVA. Data are expressed as mean ± SEM.

but not the A-component, of WDR neurons to graded intracutaneous electrical stimuli (fig. 3, B–D, n = 13). The stimulus-response functions of C-component (fig. 3D), but not A-component (fig. 3C), were significantly inhibited by DALDA in WDR neurons. We separated A-component into Aβ- (0 to 25 ms) and Aδ-mediated responses (25 to 100 ms) based on the calculated conduction velocities. The total numbers of Aβ- and Aδ-mediated responses to graded electrical stimuli (0.1 to 10 mA) after DALDA treatment (Aβ: 72.1 ± 5.3; Aδ: 29.9 ± 6.3) were not significantly different from predrug baseline (Aβ: 79.2 ± 4.9; Aδ: 29.3 ± 6.8, $P > 0.05$, paired t test). Saline treatment (n = 10) did not affect WDR neuron response (fig. 3B).

Repetitive electrical stimuli that activate C-fibers may induce temporal summation and transiently enhance the excitability of dorsal horn neurons, a phenomenon called windup (0.5 Hz, fig. 3A). Windup is most prominent in C-fiber–mediated responses of WDR neurons. Total C-component to windup stimuli was significantly decreased at 30 to 45 min after systemic administration of DALDA (10 mg/kg, intraperitoneally; fig. 3B), compared with that at predrug baseline. DALDA also significantly inhibited the windup function (fig. 3E). Saline treatment (n = 10) did not significantly change the A- or C-component of WDR neuronal response to graded electrical stimuli (fig. 3B) or the windup function (data not shown). Importantly, pretreatment with systemic methylnaltrexone (5 mg/kg, intraperitoneally, 15-min pretreatment, n = 9) completely blocked systemic DALDA-induced inhibition of C-component and windup (fig. 3, B–E). These *in vivo* electrophysiologic findings suggest that systemic administration of DALDA inhibits the C-component of WDR neurons predominantly and the A-component to a much lesser extent in SNL rats. Further, the inhibition of C-component by systemic DALDA likely occurs through the activation of peripheral opioid receptors.

Mechanical hypersensitivity is a characteristic manifestation of neuropathic pain.[27–29] WDR neurons responded to brush stimuli and showed increased firing rates to increasing intensities of punctuate mechanical stimuli (fig. 4, A–C). Although systemic DALDA inhibited mechanical allodynia, which is considered to be mediated by A-fibers, the average responses of WDR neurons to brush stimulation and the stimulus-response functions to graded punctuate mechanical stimuli were not significantly changed after systemic DALDA treatment (10 mg/kg, intraperitoneally, n = 13) compared with predrug baseline (fig. 4B). Yet, individual WDR neurons responded differently to DALDA treatment. Of 13 neurons, 6 showed a total response to graded mechanical stimuli that was decreased to less than 74% of predrug level, which is more than 2 SD less than the mean value after saline treatment (122.2 ± 24.1%, mean ± SD, n = 8; fig. 4D).Yet, two neurons paradoxically showed an increase in response to more than 170% of predrug level. Thus, quantitative mechanical testing showed different patterns of response to systemic DALDA treatment in WDR neurons of SNL rats. Saline treatment did not affect WDR neuronal response to mechanical stimulation (fig. 4C; n = 8).

DALDA Differentially Affects the Excitation of TRPV1- and MrgD-expressing DRG Neurons

Recent studies have suggested that activation of TRPV1-expressing and MrgD-expressing DRG neurons is critical to heat and mechanical pain signaling, respectively.[30–33] Therefore, we examined whether the modality preference we observed in our behavioral tests after systemic DALDA administration is paralleled by differential effects on the excitation of these two subpopulations of primary sensory neurons. We performed calcium imaging studies to examine the effects of DALDA on the $[Ca^{2+}]$ increase induced by capsaicin, which activates $TRPV1^+$ neurons, and β-alanine, which activates $MrgD^+$ neurons (fig. 5, A and B). Bath application of capsaicin (0.5 μM) increased $[Ca^{2+}]$ in 41% of DRG neurons, and β-alanine (1 mM) increased $[Ca^{2+}]$ in 17% of DRG neurons (n = 300; fig. 5C). Only 4% of DRG neurons responded to both capsaicin and β-alanine, suggesting very small colocalization of TRPV1 and MrgD in DRG neurons. After a 10-min washout of the first drug, a second application of capsaicin or β-alanine produced only a slightly smaller increase in $[Ca^{2+}]$, suggesting minimal desensitization. However, pretreatment with DALDA (10 min, bath application) dose-dependently blocked the $[Ca^{2+}]$ increase to the second application of capsaicin and β-alanine (fig. 5D). Importantly, MPEs for 0.5 and 1 μM DALDA to inhibit the capsaicin-induced $[Ca^{2+}]$ increase were significantly higher than those to inhibit the β-alanine–induced increase (fig. 5D). The IC_{50} of DALDA to inhibit the capsaicin-induced increase in $[Ca^{2+}]$ was 0.14 μM, whereas that to inhibit β-alanine–induced activation was 1.33 μM ($P < 0.01$, Student's t test). Thus, DALDA preferentially inhibits the increase in $[Ca^{2+}]$ evoked by capsaicin in DRG neurons.

TRPV1 is highly expressed in peptidergic DRG neurons, whereas MrgD is found mostly in the nonpeptidergic subpopulation. IB4 is a histochemical marker for nonpeptidergic DRG neurons, which can be labeled with IB4-fluorescein isothiocyanate in culture. Bath application of KCl (30 mM), which strongly depolarizes the cell membrane, induced a robust increase in $[Ca^{2+}]$ in both $IB4^+$ and $IB4^-$ neurons loaded with Fura-2-acetomethoxyl ester (fig. 6, A–C). As a control, sequential treatment with KCl caused only a modest reduction in the $[Ca^{2+}]$ increase. Importantly, DALDA (n = 57), but not vehicle (n = 56), significantly inhibited the KCl-evoked $[Ca^{2+}]$ increase in $IB4^-$ neurons (fig. 6D), which are likely peptidergic cells. However, in $IB4^+$ neurons, DALDA (n = 62) was no more effective than vehicle (n = 29).

Systemic DALDA Retains the Ability to Inhibit Mechanical Hypersensitivity in Nerve-injured Rats Pretreated with Resiniferatoxin

To further delineate the role of $TRPV1^+$ neurons in DALDA-induced amelioration of mechanical and thermal

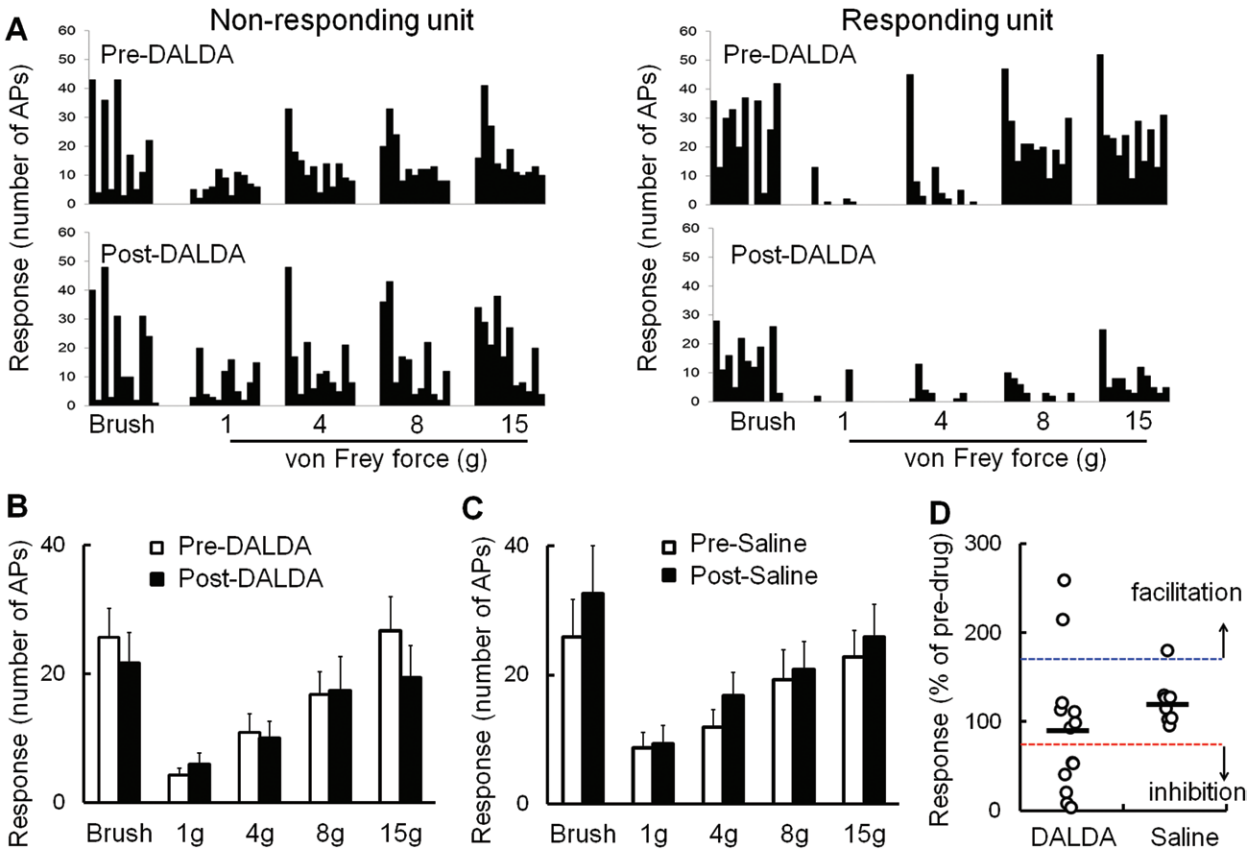

Fig. 4. Effects of systemic dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA) on the responses of wide-dynamic range (WDR) neurons to mechanical stimulation in nerve-injured rats. (*A*, *left*) Peristimulus time histograms (bin size: 0.2 s) show an example of WDR neuronal response to punctuate mechanical stimuli (1.0 to 15.0 g von Frey probe, 5 s, applied to the skin receptive field) that did not change after subcutaneous injection of DALDA (10 mg/kg, nonresponding). (*Right*) An example of another WDR neuron that shows decreased response after DALDA treatment (responding). (*B*) At 2 to 3 weeks after spinal nerve ligation (SNL) in male rats, the response to brushing stimuli and the stimulus-response functions of WDR neuronal response to graded mechanical stimuli were not significantly different from predrug baseline at 30 to 45 min after DALDA injection (10 mg/kg, subcutaneous, n = 13). (*C*) Injection of saline did not change WDR neuronal response to mechanical stimuli in SNL rats (n = 8). (*D*) The total response of each WDR neuron to graded mechanical stimuli was plotted for each group (as % predrug value). Inhibitory cells were defined as neurons that had a postdrug response that was less than 74% of predrug level (below *red dashed line*), which is more than 2 SD less than the mean of the saline group (122 ± 24%, mean ± SD, n = 8). The facilitatory cells were defined as neurons that had a postdrug response more than 170% of predrug level (above *blue dashed line*). *Black bar*: mean response. Data are expressed as mean ± SEM. AP = action potential.

hypersensitivity in nerve-injured rats, we injected adult male rats 7 days post-SNL with resiniferatoxin (0.1 mg/kg, intraperitoneally, n = 9), a highly potent and selective TRPV1 agonist that desensitizes TRPV1 receptor and decreases the excitability of TRPV1-expressing neurons. At 7 to 9 days after resiniferatoxin treatment, heat hypersensitivity was abolished in SNL rats (fig. 7A). Systemic resiniferatoxin, but not vehicle (data not shown), further induced heat analgesia, as indicated by increases in PWLs of both injured and uninjured hind paws to levels greater than the preinjury baseline (fig. 7A). However, resiniferatoxin treatment had no effect on nerve injury–induced mechanical hypersensitivity. Importantly, systemic DALDA (10 mg/kg, subcutaneously) was still able to inhibit mechanical hypersensitivity in resiniferatoxin-treated SNL rats, as indicated by a significant increase in PWT at 15 to 120 min postinjection (fig. 7B, n = 9). These data clearly indicate that systemic DALDA has different cellular targets for inhibiting nerve injury–induced mechanical and thermal hypersensitivity.

Systemic DALDA Does not Cause Opioid-related Side Effects

Finally, to determine whether systemic DALDA produces well-known opioid-related side effects such as motor incoordination and locomotor impairment, we compared male SNL rats treated with DALDA with those treated with morphine. In the rota-rod test, the fall time of rats treated with systemic DALDA (10 and 20 mg/kg, subcutaneously) was not significantly decreased at 45 min after injection compared with pre-DALDA baseline and that in saline-treated rats (fig. 8, A and B, n = 5 to 8/group). In contrast, rats treated with morphine (10 mg/kg, subcutaneously, n = 8; fig. 8B) showed a

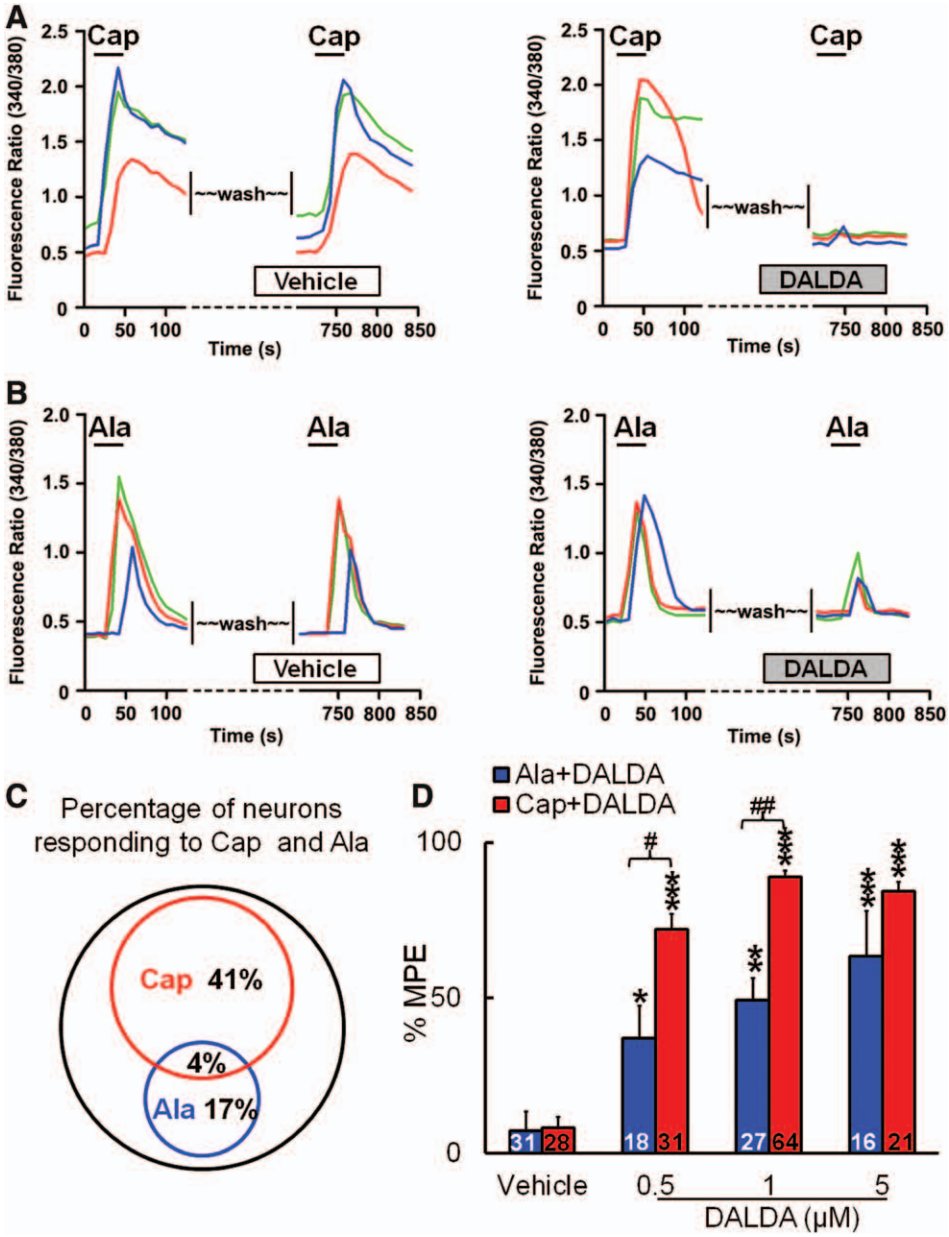

Fig. 5. Dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA) inhibits capsaicin- and β-alanine–induced increases in [Ca2+] in dorsal root ganglion (DRG) neurons. (*A, Left*) Representative traces from calcium imaging assays show the increase of [Ca^{2+}] in cultured DRG neurons in response to the first and second bath application of capsaicin (Cap; 0.5 μM). Neurons were washed (10 min) with vehicle before the second Cap application. (*Right*) Pretreatment with DALDA (1 μM, 10 min, bath application) blocked the increase in [Ca^{2+}] induced by the second application of Cap. (*B, Left*) Representative traces from calcium imaging assays show the increase of [Ca^{2+}] in cultured DRG neurons in response to the first and second bath application of β-alanine (Ala, 1 mM). Neurons were washed with vehicle before the second Ala application. (*Right*) Pretreatment with DALDA (1 μM, 10 min) partially reduced the increase in [Ca^{2+}] induced by the second application of Ala. (*C*) The Venn diagram of calcium responses illustrates the proportion of Cap- and Ala-responsive DRG neurons (n = 300). The sizes of the circles are proportional to the sizes of the cell populations. (*D*) Quantification of calcium imaging assays. DALDA (0.5, 1, and 5 μM) dose-dependently inhibited Cap- and Ala-induced increases in [Ca^{2+}]. MPE (%) = [(Pre-DALDA) − (Post-DALDA)]/(Pre-DALDA)] × 100. Numbers of neurons in each group are indicated. **P* < 0.05, ***P* < 0.01,****P* < 0.001 *versus* vehicle group; #*P* < 0.05, ##*P* < 0.01 *versus* Ala-DALDA group, one-way ANOVA. Data are expressed as mean ± SEM. MPE = maximum possible effect.

significant reduction in fall time. In additionally, in the open field test, total distance traveled (in 10 min; fig. 8, C and D), number of center crossings (fig. 8E), mean travel speed, and number of entries at the border and internal periphery (data not shown) were unaffected at 45 min after DALDA treatment (10 mg/kg, subcutaneously) compared with those at predrug baseline and in the saline-treated group (n = 5 to 8/group). Thus, nerve-injured rats displayed normal activity

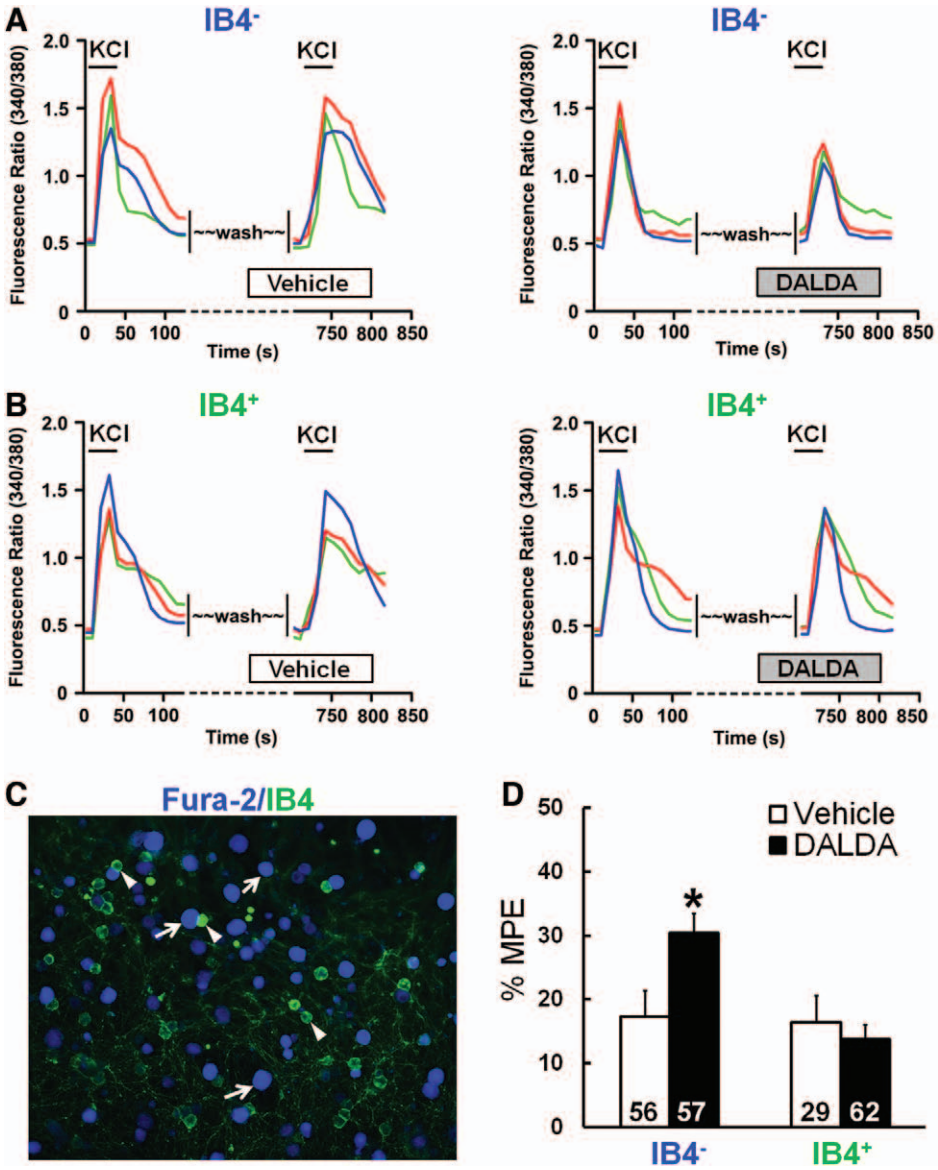

Fig. 6. Dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA) inhibits potassium chloride (KCl)-induced increases in $[Ca^{2+}]$ in isolectin IB4$^-$ dorsal root ganglion (DRG) neurons. (*A*, *left*) Representative traces from calcium imaging assays show the increase of $[Ca^{2+}]$ in isolectin IB4$^-$ DRG neurons after the first and second bath application of KCl (30 mM). Neurons were washed with vehicle (10 min) before the second KCl application. (*Right*) The $[Ca^{2+}]$ increase in response to the second application of KCl was reduced in IB4$^-$ neurons by pretreatment with DALDA (1 μM, 10 min, bath application). (*B*) DALDA did not reduce the KCl-evoked increase in $[Ca^{2+}]$ in IB4$^+$ neurons. (*C*) Fluorescent image of DRG neurons after they were labeled with IB4-fluorescein isothiocyanate and loaded with Fura-2-acetomethoxyl ester. Examples of IB4$^-$ (*purple*) and IB4$^+$ (*green*) neurons are marked with *arrows* and *arrowheads*, respectively. (*D*) Quantification of calcium imaging assays. DALDA significantly inhibited the KCl-induced increase in $[Ca^{2+}]$ in IB4$^-$ neurons, but not in IB4$^+$ neurons. MPE (%) = [(Pre-DALDA) – (Post-DALDA)]/(Pre-DALDA)] × 100. Data are expressed as mean ± SEM. *$P < 0.05$ *versus* vehicle group, Student's *t* test. MPE = maximum possible effect.

level, gross locomotion, and exploration habits after systemic DALDA treatment. In contrast, all activities were markedly reduced in morphine-treated rats (10 mg/kg, subcutaneously, n = 5/group) compared with pretreatment baseline and saline- and DALDA-treated groups.

Discussion

The therapeutic utility of centrally penetrating MOR agonists in neuropathic pain treatment is limited by central adverse effects. In this study, we utilized various behavioral, pharmacologic, *in vivo* electrophysiologic, and molecular biologic tools to demonstrate the peripherally restricted, modality-preferred antihyperalgesic effects of systemic DALDA. We further delineated potential underlying cellular mechanisms that will help to establish the therapeutic utility of peripherally acting opioids for the treatment of neuropathic pain.

Our *in vivo* electrophysiologic recordings revealed that DALDA predominantly inhibited C-fiber inputs, which

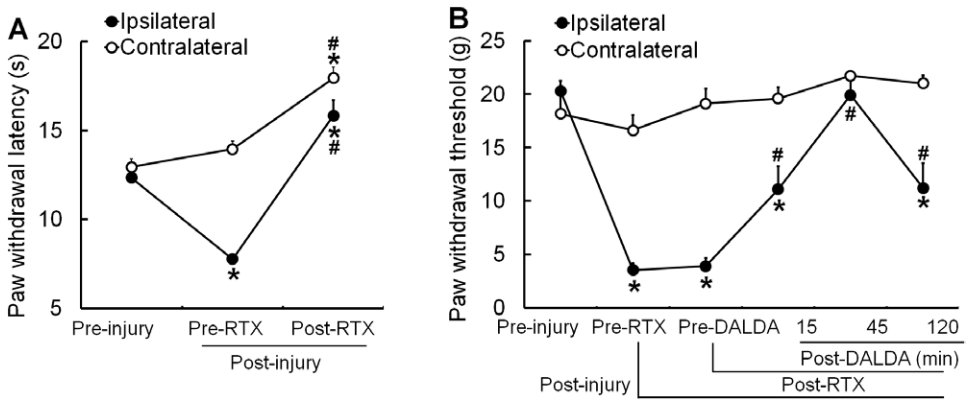

Fig. 7. Systemic dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA) retains ability to inhibit mechanical hypersensitivity in nerve-injured rats that receive resiniferatoxin (RTX). (*A*) The paw withdrawal latency (PWL) of the ipsilateral hind paw was significantly decreased in male rats on day 7 after spinal nerve ligation (SNL; pre-RTX). On days 7 to 9 after intraperitoneal injection of RTX (0.1 mg/kg, n = 9), both ipsilateral and contralateral PWLs were significantly increased from the pre-RTX and preinjury level. (*B*) Rats showed significant reduction in ipsilateral paw withdrawal thresholds on day 7 post-SNL. Systemic RTX treatment (0.1 mg/kg, intraperitoneally, n = 9) did not alter SNL-induced mechanical allodynia. However, systemic administration of DALDA (10 mg/kg, subcutaneously) significantly attenuated mechanical allodynia even after RTX treatment. *$P < 0.05$ *versus* preinjury, #$P < 0.05$ *versus* pre-RTX, one-way repeated measures ANOVA. Data are expressed as mean ± SEM.

signal thermal and noxious mechanical information, to WDR neurons. These findings complement those of the animal behavioral studies, which also showed that systemic DALDA more effectively inhibited heat hypersensitivity (mediated by C-fibers) than mechanical hypersensitivity (likely mediated by A-fibers). Although the A-component of WDR neurons to electrical simulation was not reduced, a subgroup of WDR neurons showed decreased responses to natural mechanical stimulation after systemic DALDA treatment. The reason for this discrepancy is unclear, but it is possible that systemic DALDA may change A-fiber conduction properties, which can be revealed when WDR neurons receive a prolonged barrage of afferent inputs produced by mechanical stimuli (5 s) but may not be observed with a short (2 ms) high-intensity electrical pulse. Systemic DALDA also inhibited windup of the C-component, reflecting a short-term neuronal sensitization to repetitive noxious inputs that occurs during natural stimulation of C-fibers.[34–36] Thus, systemic DALDA may also ameliorate the neuronal sensitization that underlies development of hyperalgesia by inhibiting peripheral noxious inputs.[37–41]

Although pharmacokinetic data are unavailable for subcutaneous DALDA administration in nerve-injured rats, our findings indicate that DALDA may not appreciably accumulate in CNS after systemic administration. Importantly, systemic DALDA-induced inhibition of both mechanical and heat hyperalgesia was blocked by systemic methylnaltrexon but was unaffected by intrathecal CTOP, which blocks spinal MORs over a prolonged period. Because the activation of MORs on dorsal horn neurons would reduce WDR neuronal excitability and inhibit their responses to both A- and C-fiber inputs, the finding that A-component in WDR neurons was not reduced by DALDA may also imply that systemic DALDA does not activate spinal MORs. Although systemic DALDA may activate MOR in the brain regions to induce pain inhibition, the following findings suggest that DALDA inhibits neuropathic pain primarily through peripheral mechanisms in this study. Activation of MORs in CNS would induce both antihyperalgesia and antinociception. DALDA is highly specific to MOR and is 14-fold more potent than morphine.[16] However, unlike morphine, which often induces antinociception, systemic DALDA normalized heat hyperalgesia without producing antinociception (*e.g.*, PWL above preinjury baseline) in nerve-injured rats. In addition, contralateral PWT and PWL in SNL rats did not increase after systemic DALDA treatment. If DALDA had entered the CNS, it would have induced antinociception. In addition, there is also an absence of any demonstrable CNS-related side effects known to morphine in SNL rats after systemic DALDA treatment.

We further examined the mechanisms for modality-preferred pain inhibition by systemic DALDA in male SNL rats. Different peripheral and central mechanisms are involved in mechanical and heat hypersensitivity. Both animal behavioral and *in vivo* electrophysiologic findings point to a peripheral site of action for systemic DALDA. Multimodal nociceptors in the peripheral nervous system express a wide array of ion channels and receptors that transduce intense thermal, mechanical, chemical, or cold stimuli into electrical activity.[42,43] Some receptors are segregated into different subsets of DRG neurons, such as the "heat receptor" TRPV1 and the "cold receptor" TRPM8. Mas-related G-protein-coupled receptors (*e.g.*, MrgC, MrgD) are also distributed in a mutually exclusive fashion in DRG neurons.[30,33,44,45] Thus, different subpopulations of primary sensory neurons may contribute to the modality-specific differences in DALDA's effects on mechanical and thermal hyperalgesia.[30,46] Small-diameter DRG neurons, which are presumably nociceptive, generally

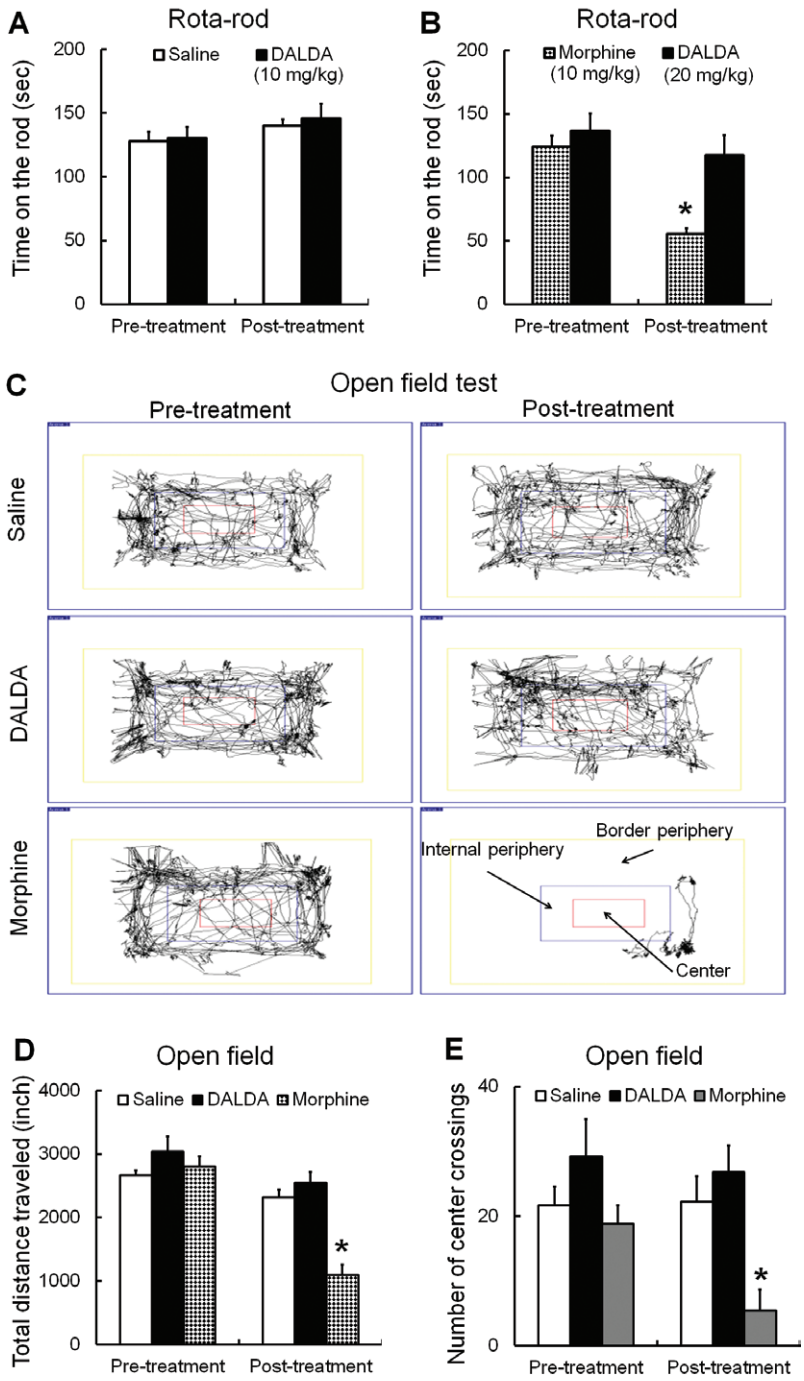

Fig. 8. Systemic dermorphin [D-Arg2, Lys4] (1–4) amide (DALDA) does not induce opioid-related side effects and does not affect exploration activity of rats. (*A*) In the rota-rod test, neither DALDA (10 mg/kg, subcutaneously, n = 5) nor saline (n = 8) decreased fall time (*i.e.*, time on the rod) in spinal nerve ligation (SNL) male rats at 45 min postinjection, compared with that at baseline. (*B*) Morphine (10 mg/kg, subcutaneously, n = 8), but not a higher dose of DALDA (20 mg/kg, n = 8), did induce motor dysfunction on the rota-rod test at 45 min postinjection. (*C*) Examples of SNL rat exploration activity (10-min duration) in the open field test before and 45 min after injection of saline, DALDA (10 mg/kg, subcutaneously), and morphine (10 mg/kg, subcutaneously, n = 5/group). (*D*, *E*) In the open field test, morphine (10 mg/kg, subcutaneously), but not DALDA (10 mg/kg, subcutaneously) or saline (n = 5/group), reduced the total distance traveled in 10 min and the number of center crossings by SNL rats at 45 min after injection. *$P < 0.05$ *versus* pretreatment, paired *t* test. Data are expressed as mean ± SEM.

can be separated into peptidergic and nonpeptidergic subpopulations. The vanilloid receptor TRPV1 is well known for its role in heat pain signaling. TRPV1 and MOR are more highly expressed and colocalized in peptidergic DRG neurons (likely $IB4^-$) than in the nonpeptidergic subpopulation. DALDA shows high binding affinity and is highly selectivity for MORs

with a selectivity ratio Ki-δ /Ki-μ of 11,400.[16–18] Therefore, DALDA may preferably inhibit heat-sensing TRPV1$^+$ neurons by activating MORs. This preference may partially explain the greater efficacy of systemic DALDA to inhibit heat hypersensitivity than mechanical hypersensitivity. In line with this notion, DALDA significantly inhibited the [Ca^{2+}] increase induced by KCl in IB4$^-$ neurons but not in IB4$^+$ neurons. Further, DALDA induced significantly greater inhibition of the [Ca^{2+}] increase evoked by capsaicin, which activates TRPV1, than that evoked by β-alanine, which activates MrgD. TRPV1$^+$ neurons play a dominant role in heat nociception and hyperalgesia, whereas MrgD$^+$ neurons are important to mechanical pain signaling.[30,31,47] MrgD is expressed mostly in nonpeptidergic neurons and rarely colocalize with TRPV1. Indeed, our results also suggest that only 4% of DRG neurons respond to both capsaicin and β-alanine. Systemic treatment with resiniferatoxin, which selectively decreases the excitability of TRPV1$^+$ neurons,[48,49] produced a prolonged reversal of heat hypersensitivity, but not mechanical hypersensitivity, in nerve-injured rats. Interestingly, resiniferatoxin treatment did not affect the attenuation of mechanical allodynia by systemic administration of DALDA. This finding suggests that systemic DALDA ameliorates mechanical and heat hypersensitivity *via* different cellular targets. Together, these findings suggest a potential cellular mechanism by which DALDA preferentially inhibits heat hypersensitivity.

Finally, to study the safety profile of DALDA, we tested DALDA-treated rats in rota-rod and open field tests. In contrast to morphine, systemic DALDA did not impair motor coordination of rats in the rota-rod test, even at the highest doses tested. In addition, although morphine-treated rats exhibited reductions in distance traveled and travel speed in the open field test, the locomotor function of DALDA-treated rats appeared unchanged from baseline. Additional findings from other investigators suggest that DALDA produces only a transient, minor increase in blood pressure and does not affect maternal respiratory, hemodynamic, or metabolic functions, further suggesting that DALDA has a minimal side-effect profile.[50] However, DALDA may share other peripheral nervous system side effects (*e.g.*, constipation, vomiting, dry mouth) known to peripherally acting μ-opioids after repetitive and long-term drug treatment. The pharmacokinetic, peripheral nervous system side effects and influences on bowel function of DALDA need to be systematic and carefully investigated in future to fully characterize the pharmacologic properties of this molecule. Our recent study suggested that repeated use of loperamide for alleviating neuropathic mechanical hypersensitivity may lead to the development of tolerance, possibly at peripheral opioid receptors.[51] Because different MOR agonists induce different magnitudes of receptor internalization, desensitization, and tolerance processes, it remains to be tested whether DALDA leads to the development of analgesic tolerance or opioid-induced hyperalgesia.

Systemic DALDA (10 mg/kg, subcutaneously) also alleviated mechanical and heat hypersensitivity in female rats after nerve injury. Intriguingly, there was a trend that MPE for DALDA to inhibit mechanical hypersensitivity in female rats was lower than that in male rats, suggesting possible gender-based differences. Because estrous cycles of female may profoundly affect pain response and drug effects,[52–54] future studies need to characterize gender difference and determine effects of estrous cycles on the efficacy and mechanisms of pain inhibition by DALDA. Such studies will help to fully establish the clinical usefulness of peripherally acting opioids for a therapeutic formulation. In summary, our findings suggest that systemic administration of DALDA attenuates both mechanical and heat hypersensitivity in nerve-injured rats through activation of MORs at peripheral but not at central sites. Further, the efficacy of DALDA to inhibit heat hypersensitivity is greater than that to inhibit mechanical hypersensitivity in male rats. Because it does not affect CNS function, DALDA may pose minimal risk for central dose-limiting adverse effects and have low addiction or abuse potential. Hence, DALDA may represent a promising therapeutic alternative to currently used opioids for the treatment of neuropathic pain.

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Competing Interests

The authors declare no competing interests.

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