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Short communication

Histone deacetylase inhibitors relieve morphine resistance in neuropathic pain after peripheral nerve injury

Hitoshi Uchida [1], Yosuke Matsushita [1], Kohei Araki, Takehiro Mukae, Hiroshi Ueda*

Department of Pharmacology and Therapeutic Innovation, Nagasaki University Graduate School of Biomedical Sciences, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

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ABSTRACT

Neuropathic pain is often insensitive to morphine. Our previous study has demonstrated that neuron-restrictive silencer factor represses mu opioid receptor (MOP) gene expression in the dorsal root ganglion (DRG) via histone hypoacetylation-mediated mechanisms after peripheral nerve injury, thereby causing loss of peripheral morphine analgesia. Here, we showed that histone deacetylase (HDAC) inhibitors, such as trichostatin A and valproic acid, restored peripheral and systemic morphine analgesia in neuropathic pain. Also, these agents blocked nerve injury-induced MOP down-regulation in the DRG. These results suggest that HDAC inhibitors could serve as adjuvant analgesics to morphine for the management of neuropathic pain.

Neuropathic pain is manifested by combination of positive (hyperalgesia and allodynia) and negative (hypoesthesia and hypoalgesia) symptoms (1). Patients with neuropathic pain are often insensitive to conventional analgesics, including morphine (2). Also, the morphine resistance has been observed in diverse animal models of neuropathic pain caused by peripheral nerve injury (3), post-herpetic neuralgia (4), and bone cancer (5). Under these models, the common event is a down-regulation of mu opioid receptor (MOP) expression in the dorsal root ganglion (DRG) (3–5). Accordingly, previous studies have shown that the intraplantarly administrated morphine fails to produce analgesic effects on neuropathic pain (3), and that injury reduces the pre-synaptic inhibitory action of MOP agonist (6). Therefore, the elucidation of mechanisms underlying MOP down-regulation after injury could be important to overcome the morphine resistance in neuropathic pain.

Neuron-restrictive silencer factor (NRSF, also known as repressor element-1 silencing transcription factor) represses transcription of neuron-restrictive silencer element (NRSE)-containing genes via recruitment of histone deactylase (HDAC) enzymes through co-repressors, mSin3 and CoREST (7). Our previous study has demonstrated that NRSF causes injury-induced morphine resistance and hypoesthesia by histone hypoacetylation-mediated silencing of NRSE-containing MOP and sodium channel $Na_v1.8$ genes, respectively (8). We have also reported that HDAC inhibitors, such as trichostatin A (TSA) and valproic acid (VPA), block injury-induced $Na_v1.8$ down-regulation in the DRG and hence improve hypoesthesia (9); yet, the functional involvement of HDAC in morphine resistance remains to be investigated. Thus, we tested here whether HDAC inhibitors could block injury-induced MOP down-regulation, thereby relieving the morphine resistance.

Male C57BL/6J mice weighing 20–25 g were used. They were kept in a room with a temperature of 21 ± 2 °C with free access to a standard laboratory diet and tap water. All experimental procedures were approved by the Nagasaki University Animal Care Committee, and complied with the fundamental guidelines for the proper conduct of animal experiments and related activities in academic research institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, Japan. Partial ligation of the sciatic nerve was performed as described previously (8).

TSA (Wako Pure Chemical Industries, Ltd., Osaka) dissolved in 10% ethanol in physiological saline and VPA sodium salt (Sigma–Aldrich, St. Louis, MO, USA) dissolved in physiological saline were intraperitoneally injected 60 min and 120 min prior to injury, respectively, and once daily post-injury. Analgesic tests were performed 60 min or 120 min after the last treatment of TSA or VPA,

* Corresponding author. Tel.: +81 95 819 2421; fax: +81 95 819 2420.
E-mail address: ueda@nagasaki-u.ac.jp (H. Ueda).
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[1] **Equal contribution**: Both authors equally contributed to this study.

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respectively. Morphine hydrochloride (Takeda Chemical Industries, Osaka) dissolved in physiological saline was injected intraplantarly or subcutaneously. To assess morphine analgesia, the area under the curve was measured by trapezoidal rule with subtraction of the basal value at 0 min from the value for each withdrawal threshold from 10 to 60 min after morphine treatment.

In thermal paw withdrawal tests, the latency to withdrawal from a thermal stimulus (IITC Inc., Woodland Hills, CA, USA) was assessed as a nociception threshold, as described previously (8). A cut-off time of 20 s was set to prevent tissue damage.

We collected L4-6 DRGs just after analgesic tests. The extraction of total RNA from L4-6 DRGs, cDNA synthesis, and real-time PCR using gene-specific primers were performed as described previously (8). Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control for normalization.

Statistical analyses of results were performed using Student's *t*-test or a one-way ANOVA with Tukey–Kramer multiple comparison *post hoc* test. The criterion of significance was set at $P < 0.05$. All results were expressed as means ± SEM.

Firstly, we confirmed that a single intraperitoneal (i.p.) administration of TSA (1 mg/kg) elevated histone H3 and H4 acetylation in the DRG at least 60 min post-injection (data not shown). To examine whether HDAC inhibitor could relieve the loss of peripheral morphine analgesia after injury, mice were given repeated i.p. injections of TSA (1 mg/kg) for 7 days, starting from 60 min prior to injury. Consistent with previous report (9), neither the basal nociceptive threshold in sham-operated mice nor thermal pain hypersensitivity in nerve-injured mice was affected by TSA (Fig. 1: A, B). Meanwhile, a lack of analgesic action of intraplantarly injected morphine (30 nmol) was remarkably recovered by TSA (Fig. 1: B, C). In contrast, TSA had no effects on peripheral morphine analgesia in sham-operated mice (Fig. 1: A, C).

Next, we assessed whether HDAC inhibitors could restore the systemic morphine analgesia after injury. Mice were treated with subcutaneous (s.c.) injections of morphine at a dose of 1 mg/kg that fails to produce analgesia in nerve-injured mice (3). The loss of systemic morphine analgesia at day 3 post-injury was recovered by TSA (Fig. 2: A–C). In addition to TSA, we used VPA, another HDAC inhibitor, at a dose of 200 mg/kg that exerts no anti-hyperalgesic action (10) but blocks injury-induced $Na_V1.8$ down-regulation in the DRG (9). Repeated i.p. treatments with VPA also restored the systemic morphine analgesia in nerve-injured mice (Fig. 2D). Moreover, VPA significantly improved the sensitivity to peripherally injected morphine, but not thermal hyperalgesia, after injury (data not shown). In contrast, both HDAC inhibitors had no effects

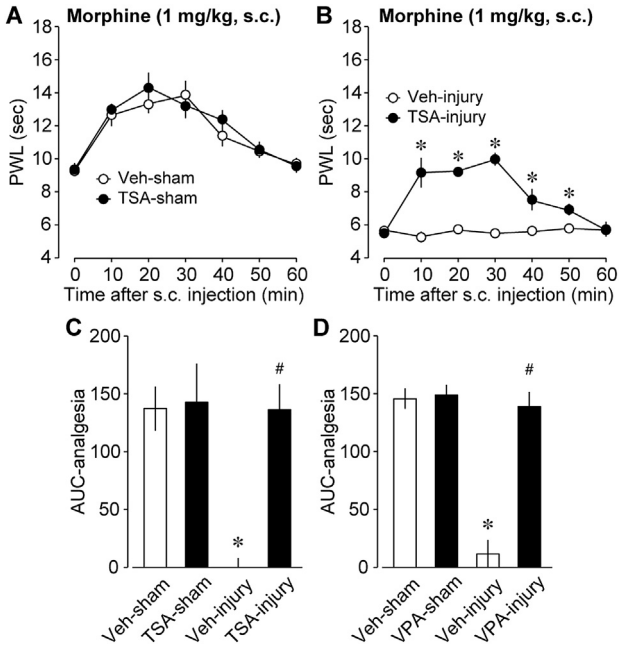

Fig. 2. Prevention of injury-induced loss of systemic morphine analgesia by HDAC inhibitors. TSA (1 mg/kg, i.p.) or VPA (200 mg/kg, i.p.) was administrated 60 min or 120 min prior to injury, respectively, and once daily for 3 days after nerve injury. Thermal pain threshold was assessed at day 3 post-injury, using a thermal paw withdrawal test. A and B) Time-courses of thermal paw-withdrawal latencies (PWL, in seconds) after morphine (1 mg/kg, s.c.) injection in sham-operated mice (A) and nerve-injured mice (B). $^*P < 0.05$, vs. vehicle (Veh)-treated group. C and D) Comparison of morphine analgesia after TSA (C) and VPA (D) treatments by area under the curve (AUC). $^*P < 0.05$, vs. Veh-treated and sham-operated group and $\#P < 0.05$, vs. Veh-treated and nerve-injured group. Data are expressed as the means ± SEM from at least five mice.

on the systemic morphine analgesia in sham-operated mice (Fig. 2: A, C, D).

To test whether HDAC inhibitors could block injury-induced MOP down-regulation, we isolated L4-6 DRGs at day 3 post-injury, and mRNA expression levels were quantified by real-time PCR. Injury-induced reduction of MOP mRNA expression was significantly recovered by treatments with TSA and VPA (Fig. 3: A, B). In contrast, these agents had no effects on MOP expression in sham-operated mice, in agreement with the behavioural data (Fig. 2).

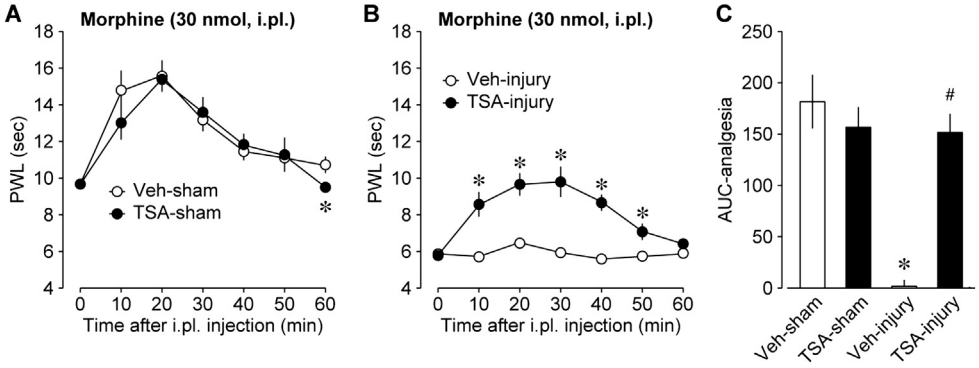

Fig. 1. Blockade of injury-induced loss of peripheral morphine analgesia by TSA. TSA (1 mg/kg, i.p.) was administrated 60 min prior to injury and once daily for 7 days after nerve injury. Thermal pain threshold was assessed at day 7 post-injury, using a thermal paw withdrawal test. A and B) Time-courses of thermal paw-withdrawal latencies (PWL, in seconds) after intraplantar injection of morphine (30 nmol) in sham-operated mice (A) and nerve-injured mice (B). $^*P < 0.05$, vs. vehicle (Veh)-treated group. C) Comparison of morphine analgesia by area under the curve (AUC). $^*P < 0.05$, vs. Veh-treated and sham-operated group and $\#P < 0.05$, vs. Veh-treated and nerve-injured group. Data are expressed as the means ± SEM from at least ten mice.

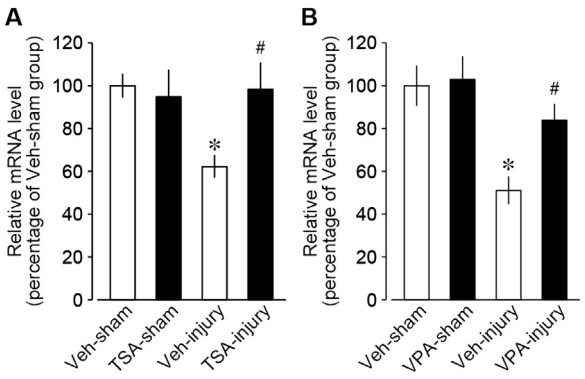

Fig. 3. Blockade of injury-induced MOP down-regulation by HDAC inhibitors. TSA (1 mg/kg, i.p.) or VPA (200 mg/kg, i.p.) was administrated 60 min or 120 min prior to injury, respectively, and once daily for 3 days after nerve injury. A and B) The effects of TSA (A) and VPA (B) on nerve injury-induced down-regulations of MOP mRNA in the DRG at day 3 post-injury. The mRNA expression levels were quantified by real-time PCR, and normalized to that of GAPDH mRNA. Results are calculated as percentages of vehicle (Veh)-treated and sham-operated group, and expressed as means ± SEM from at least eight mice. $^{*}P < 0.05$, vs. Veh-treated and sham-operated group and $\#P < 0.05$, vs. Veh-treated and nerve-injured group.

NRSF is known to target MOP gene that contains NRSE sequence around its initial codon (11). In neuronal cells, NRSF-mediated repression of MOP expression is sensitive to TSA, suggesting that HDAC is needed for silencing of MOP gene via NRSF (11). Previously, we have demonstrated that injury up-regulates NRSF expression in the DRG, concomitantly with an increase of NRSF binding to MOP–NRSE and a decrease in histone acetylation at the same site (8). Importantly, antisense knockdown of NRSF blocks injury-induced MOP down-regulation in the DRG, indicating a causal role of NRSF (8). Here, we showed that injury-induced MOP down-regulation was recovered by the treatments with HDAC inhibitors. However, these agents were unable to affect MOP expression in the sham-operated mice, in agreement with the findings obtained from antisense-mediated NRSF knockdown (8). These findings suggest that injury could initiate HDAC-mediated mechanisms underlying MOP down-regulation in the DRG, possibly inducing NRSF expression. Based on the evidence that TSA has no effects on NRSF expression in neuronal cells (11) and that injury-induced NRSF up-regulation in the DRG is insensitive to HDAC inhibitor suberoylanilide hydroxamic acid (9), it is unlikely that HDAC inhibitors prevent injury-induced MOP down-regulation via blockade of NRSF induction in the DRG. On the other hand, NRSF has been known to preferentially recruit HDAC1 and HDAC2 via mSin3 and CoREST (7). Previous study has shown that transcription factor Sp3 binds to GC-box adjacent to MOP–NRSE and interacts with NRSF and HDAC2, but not HDAC1, to synergistically repress MOP transcription (12). Further studies are required to identify the HDAC subtypes involved in NRSF-mediated silencing of MOP transcription after injury. Also, whether HDAC inhibitors restore MOP expression after injury via blockade of NRSF–Sp3–HDAC2-mediated epigenetic mechanisms is the subject of future research.

Previously, we have shown that subcutaneously, intraplantarly and intrathecally, but not intracerebroventricularly, administrated morphine exert diminished analgesic effects after injury (3), suggesting that the impairments of peripheral and spinal morphine actions are responsible for the reduced efficacy of systemic morphine. Accordingly, electrophysiological study has revealed that injury reduces spinal pre- and post-synaptic actions of MOP agonist (6). Importantly, mice lacking G protein-coupled inwardly rectifying potassium channel 2, a major post-synaptic effector of morphine, show significant residual morphine analgesia, implying a critical contribution of pre-synaptic morphine action to its analgesic effect (13). Given that HDAC inhibitors blocked injury-induced MOP down-regulation in the DRG, it is conceivable that HDAC inhibitors could ameliorate peripheral and spinal pre-synaptic actions of morphine, thereby restoring peripheral and systemic morphine analgesia.

Adjuvant analgesics include anticonvulsant agents, such as VPA that potentiates morphine analgesia (14), possibly through inhibition of gamma-aminobutyric acid (GABA) transaminase. Considering that VPA at a dose of 200 mg/kg failed to affect morphine analgesia in sham-operated mice, it is improbable that VPA could restore morphine analgesia after injury via an increase in GABA concentration. Alternatively, the present study suggested that VPA could reduce the dose of morphine required for the management of neuropathic pain via the restoration of MOP expression in the DRG. Additionally, VPA has been reported to prevent the development of analgesic tolerance to morphine through inhibition of glycogen synthase kinase 3β (15). Collectively, these observations suggest that VPA has multiple benefits in the treatment of neuropathic pain with morphine.

In summary, we show here that HDAC inhibitors block injury-induced MOP down-regulation in the DRG, thereby restoring peripheral and systemic morphine analgesia. Thus, HDAC inhibitors might be a novel adjuvant analgesic to morphine for the management of neuropathic pain.

Conflict of interest

The authors indicated no potential conflicts of interest.

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