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ORL_1 Activation Mediates a Novel ORL_1 Receptor Agonist SCH221510 Analgesia in Neuropathic Pain in Rats

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Abstract

Opioid receptor like 1 (ORL_1) receptor activation displayed an anti-nociceptive effect at spinal level for acute and neuropathic pain. SCH221510, an orally active non-peptide ORL_1 agonist, was reported to be effective in treating neuropathic pain. The present study used ORL_1 antagonist and siRNA to investigate that ORL_1 activation mediates intrathecal SCH221510 analgesia in neuropathic pain induced by chronic constrictive injury (CCI) to rat sciatic nerve. Paw withdrawal latency and 50% mechanical threshold were measured for thermal and mechanical hypersensitivity in rats. CCI significantly decreased paw withdrawal latency and mechanical threshold. SCH221510 (3, 10, 30 μg) or ORL_1 antagonist ([Nphe^1]nociceptin(1-13)NH_2, 10 μg) was intrathecally injected to test the behavioral effects on neuropathic pain. Intrathecal siRNA was started on 1 day before CCI surgery and maintained for 7 days. L4-L5 spinal cord ORL_1 mRNA and protein were measured by real-time PCR and Western blot. The effect of intrathecal siRNA on SCH2210510 was tested in CCI rats on day 7. Intrathecal SCH221510 dose-dependently reduced thermal and mechanical hypersensitivity induced by CCI. [Nphe^1]nociceptin(1-13)NH_2 blocked SCH221510 analgesia in CCI rats. Intrathecal siRNA blocked ORL_1 mRNA and protein increase induced by CCI. Intrathecal ORL_1 siRNA did not change thermal and mechanical hypersensitivity induced by nerve injury. Intrathecal siRNA blocked SCH221510 analgesia in neuropathic pain at spinal level. Conclusively, ORL_1 activation mediates SCH221510 analgesia in neuropathic pain at spinal level. The results warrant a potential clinically applicable drug in treating neuropathic pain.

Keywords ORL_1 · CCI · SCH221510 · [Nphe^1]nociceptin(1-13)NH_2 nociceptin/Orphanin FQ

Introduction

Neuropathic pain is a debilitating chronic condition developed after nerve injury that patients suffering from neuropathic pain are hypersensitive to pain stimuli. The plastic changes in nociceptive neurons and central nervous descending modulatory pathways maintain the chronic status of neuropathic pain (Cohen & Mao, 2014). Neuropathic pain is refractory to treatments and severely affecting life quality of patients suffering these symptoms. There are debates on the effectiveness of neuropathic pain treatments and clinical studies have shown that limited efficacy and poor phenotypic profiling probably account for modest treatment outcomes (Finnerup, et al., 2015; Truini, 2017). In addition, morphine and other mu-opioid peptide receptor agonists have been used in treating peripheral nerve-injury-induced neuropathic pain but effects are complicated due to the side effects including abuse and addiction (Dowell, Haegerich, & Chou, 2016).

Opioid receptor like 1 (ORL_1) receptor, one of the opioid receptor family, was found to bind to its natural ligand nociceptin/orphanin FQ (N/OFQ) but not to the classical opioid receptor agonists (Mogil & Pasternak, 2001). The analgesic effect of ORL_1 activation depended on administration sites. Reports agreed that high doses of N/OFQ produced antinociceptive and anti-allodynia effects (Courteix, et al., 2004; Wang, Zhu, Cao, & Wu, 1999), although studies have reported that supraspinal injection of N/OFQ could produce pronociceptive or analgesic effects (Mogil, et al., 1996; Standifer, et al., 1996; Tian, et al., 1997). Variety of ORL_1

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agonists have been developed to decrease nerve-injury-induced thermal hypersensitivity and mechanical allodynia through spinal level administration (Obara, Przewlocki, & Przewlocka, 2005; Rizzi, et al., 2015). SCH221510 is a potent and selective non-peptide ORL_1 agonist which provided the antinociceptive effect in a rodent model of inflammatory bowel disease with an oral active property (Sobczak, et al., 2014; Varty, et al., 2008). In addition, SCH221510 showed its analgesic effects on acute pain in primates and neuropathic pain in mice (Cremeans, Gruley, Kyle, & Ko, 2012; Sukhtankar, Zaveri, Husbands, & Ko, 2013), suggesting a possible therapeutic effect in neuropathic pain treatment. However, the mechanism is still elusive whether spinal ORL_1 mediates SCH221510 analgesia in neuropathic pain. The present study utilized ORL_1 receptor antagonist [$Nphe^1$]nociceptin(1-13)NH_2 and ORL_1 siRNA to investigate ORL_1 activation antinociceptive effect in sciatic nerve chronic constrictive injury (CCI) rats.

Methods and Materials

Animals

Male Sprague-Dawley rats (Experimental Animal Center, Luoyang Medical College, Luoyang, Henan, China) weighing 200–220 g were used. Rats were acclimated for 1 week before experiments. The experiment was approved by the Animals Care and Use Committee of Luoyang Medical College and conformed to the guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

Surgery

The chronic constrictive injury to sciatic nerve was induced by four loose ligatures as previously described (Bennett & Xie, 1988). Under sodium pentobarbital anesthesia, left sciatic nerve was exposed and four 4-0 chronic gut sutures were tied loosely around the nerve. The incision was sutured up and rat was allowed to recover from anesthesia. The surgery was performed under sterile techniques and antibiotic ointment was applied to prevent postoperative infection. Hind paw thermal and mechanical hypersensitivity developed after CCI.

Cannulation and siRNA Administration

Intrathecal catheter was placed 3 days before nerve ligation. A PE-10 tube was inserted through the gap between vertebrae L5 and L6 and extended 1.5 cm into the subarachniod space of lumbar enlargement. The catheter was sealed at the end and embedded under the neck skin. The surgery was performed under sterile techniques and antibiotic ointment was applied to prevent postoperative infection. The cannulated rat was housed individually and allowed to recover for 3 days prior behavioral tests. One day before CCI surgery, ORL_1 siRNA was intrathecally injected. Over a period of 1 min, 30 μl of sterile buffer (containing 3 μl of 20 μM siRNA (MSS276173); 12 μl oligofectamine, ThermoFisher Scientific, Grand Island, and 15 μl sterile buffered saline solution) was injected through the cannula using a Hamilton syringe. siRNA was daily injected for 7 days (Baker, Chen, Shah, & Okuse, 2011).

Behavioral Tests

Thermal and mechanical hypersensitivity were measured by paw withdrawal latency (PWL) to heat and 50% mechanical threshold (mechanical threshold calculated by up-down method with filaments of different weights) to von Frey filament on hind paws. Rats were acclimated in enclosure on a glass table for 15 min. PWL to radiant heat was measured as thermal sensitivity using IITC Model 336 Tail Flick Analgesia Meter (Life Science Instrument, Cridersville, OH, USA). The radiant heat was applied to plantar of hind paws and time between heat start and hind paw withdrawal was recorded as PWL. The radiant heat intensity was adjusted to produce a stable withdrawal latency around 10 s in naive rats with a 20-s cutoff to avoid tissue damage. Fifty percent mechanical threshold to von Frey filaments was measured as mechanical sensitivity. Rats were placed on the metal mesh floor in enclosure with 15 min acclimation. von Frey filaments (0.25, 0.65, 1.05, 1.56, 2.60, 4.89, 6.16, 8.40, 15.25, and, 21.75 g, Stoelting, Wood Dale, IL, USA) were pressed onto plantar surface, starting from 6.16 g in an up-and-down method. Next stronger filament was applied upon positive response and next lower one was used after negative response, and then 50% paw withdrawal threshold was calculated. All the behaviors were tested before CCI and on days 3, 7, 10, and 14 after CCI. Specifically, SCH221510 effects were tested at 10, 20, 30, 60, and 90 min after the drug administration on day 7. Ten microliters of SCH221510 (3, 10, and 30 μg, dissolved in 10% DMSO in saline) or ORL_1 antagonist [$Nphe^1$]nociceptin(1-13)NH_2 (10 μg, dissolved in 10% acetonitrile in saline) (Tocris, Minneapolis, MN, USA) was injected through intrathecal catheter.

On day 7 after CCI, rats were decapitated and the L4-L5 segments of the spinal cord were removed. Dorsal part of the spinal cord was frozen on dry ice and kept at − 80 °C.

Real-Time PCR

Total RNA was extracted from the spinal cord tissue of untreated, CCI, and CCI with siRNA groups by Trizol (15596026, ThermoFisher Scientific, Grand Island, NY). Tissue DNA was removed by DNase, and then RNA was reversely transcribed to cDNA (A3500, Promega, Madison, WI). SYBR green (4472903, ThermoFisher Scientific, Grand Island, NY) was utilized with primers (ORL_1 (OPRL1),

PPR44465A; Rn18s, PPM72041A, Qiagen, Germantown, MD) to quantify levels of ORL_1 mRNA in the spinal cord of those three groups; 18 s was used as an internal control.

Western Blotting

Total protein was extracted from the spinal cord tissue of untreated, CCI, and CCI with siRNA. Protein samples were separated using a 4–15% Mini-PROTEAN TGX precast polyacrylamide gel (Bio-Rad, Hercules, CA). After electrophoresis, separated proteins were transferred to a polyvinylidene difluoride membrane. ORL_1 protein bands were visualized using a rabbit polyclonal primary antibody (ab66219; Abcam, Cambridge, MA; 1:1000), along with GAPDH as an internal control (A00915, GenScript, Piscataway, NJ; 1:2500), a biotinylated anti-rabbit secondary antibody (Vector Labs, Burlingame, CA; 1:400), streptavidin-HRP conjugates, and chemiluminescent substrate (Pierce, Rockford, IL). Band densitometry was performed using ImageJ (NIH, Bethesda, MD).

Statistics

Data are presented as mean ± SEM and analyzed by Prism 4.0. Repeated measures analysis of variance (ANOVA) followed by Student–Newman–Keuls test was used for post hoc analysis for differences between groups. $p < 0.05$ was considered statistically significant.

Results

Effects of Intrathecal SCH221510 and ORL_1 Antagonist

The PWL and 50% mechanical threshold were significantly decreased 3 days after CCI injury when compared with baseline. The effects of intrathecal SCH221510 (3, 10, and 30 μg, $n = 7–9$) on PWL and 50% mechanical threshold were tested on day 7 after CCI. There were significant increases in PWL at 20 min ($F = 4.013$, $p < 0.05$), 30 min ($F = 6.823$, $p < 0.01$), 60 min ($F = 10.522$, $p < 0.001$), and 90 min ($F = 5.631$, $p < 0.01$) in 10 and 30 μg SCH221510 groups but not at 10 min ($p > 0.05$, Fig. 1a). No significant change was detected in 3 μg SCH221510 group ($p > 0.05$, compared with solvent). There were also significant increases in 50% mechanical threshold at 20 min ($F = 5.513$, $p < 0.05$), 30 min ($F = 7.921$, $p < 0.01$), 60 min ($F = 12.124$, $p < 0.001$), and 90 min ($F = 6.138$, $p < 0.05$) in 10 and 30 μg SCH221510 groups but not at 10 min ($p > 0.05$, Fig. 1b). No significant change was detected in 3 μg SCH221510 group ($p > 0.05$, compared with solvent).

Intrathecal of ORL_1 antagonist, [$Nphe^1$]nociceptin(1-13)NH_2 (10 μg), alone did not change either PWL or 50% mechanical threshold. Intrathecal 10 μg SCH221510 significantly increased both PWL ($p < 0.05$, 0.01, 0.001, and 0.01, Fig. 1c) and 50% mechanical threshold ($p < 0.05$, 0.01, 0.001, and 0.05, Fig. 1d) at 20, 30, 60, and 90 min as described above. When 10 μg SCH221510 and 10 μg [$Nphe^1$]nociceptin(1-13)NH_2 were intrathecally injected concurrently, [$Nphe^1$]nociceptin(1-13)NH_2 blocked SCH22150 analgesic effect on both PWL and 50% mechanical threshold ($p < 0.05$, 0.01, Fig. 1c, d). There were no differences in [$Nphe^1$]nociceptin(1-13)NH_2 plus SCH221510 group when compared with time point 0 min on PWL and 50% mechanical threshold ($p > 0.05$, Fig. 1c, d).

ORL_1 siRNA Blocked ORL_1 mRNA and Protein Increases Induced by CCI

ORL_1 mRNA and protein from the spinal cord L4-L5 segments were measured in untreated, CCI, and CCI plus ORL_1 siRNA groups. Real-time PCR result showed that ORL_1 mRNA in CCI rats was 4.19 times of that in untreated group when normalized to 18 s ($p < 0.01$). ORL_1 siRNA significantly blocked nerve-injury-induced ORL_1 mRNA increase when compared to CCI group ($p < 0.05$) (Fig. 2a). ORL_1 protein levels in the spinal cord L4-L5 segments were measured by Western blot. The result showed that nerve injury increased ORL_1 protein ($p < 0.01$, CCI vs. untreated) and ORL_1 siRNA blocked CCI-induced ORL_1 protein increase ($p < 0.05$, CCI plus ORL_1 siRNA vs. CCI) (Fig. 2b). All ORL_1 protein bands were normalized to GAPDH.

ORL_1 siRNA Blocked SCH221510 Analgesia

SCH221510 was intrathecally injected daily for 7 days starting from 1 day before CCI surgery. The effects of intrathecal ORL_1 siRNA on CCI-induced thermal and mechanical hypersensitivity were tested on days 3, 7, 10, and 14. ORL_1 siRNA did not change PWL and mechanical threshold on days 3, 7, 10, and 14 when compared with CCI group ($p > 0.05$ vs. CCI) (Fig. 3a, b).

Ten micrograms SCH221510 was intrathecally injected in CCI group and CCI rats treated with ORL_1 siRNA group on day 7 after CCI. SCH221510 significantly increased PWL and 50% mechanical threshold of CCI rats when compared with control (CCI only) group ($p < 0.01$) (Fig. 4a, b). However, PWL and 50% mechanical threshold increases were blocked by ORL_1 siRNA when compared with SCH2210510 treated group ($p < 0.05$) (Fig. 4a, b). There was no difference between SCH221510 plus ORL_1 siRNA group and control (CCI only) group on both PWL and mechanical threshold, indicating that SCH221510 analgesia was abolished by ORL_1 siRNA in CCI rats ($p > 0.05$) (Fig. 4a, b).

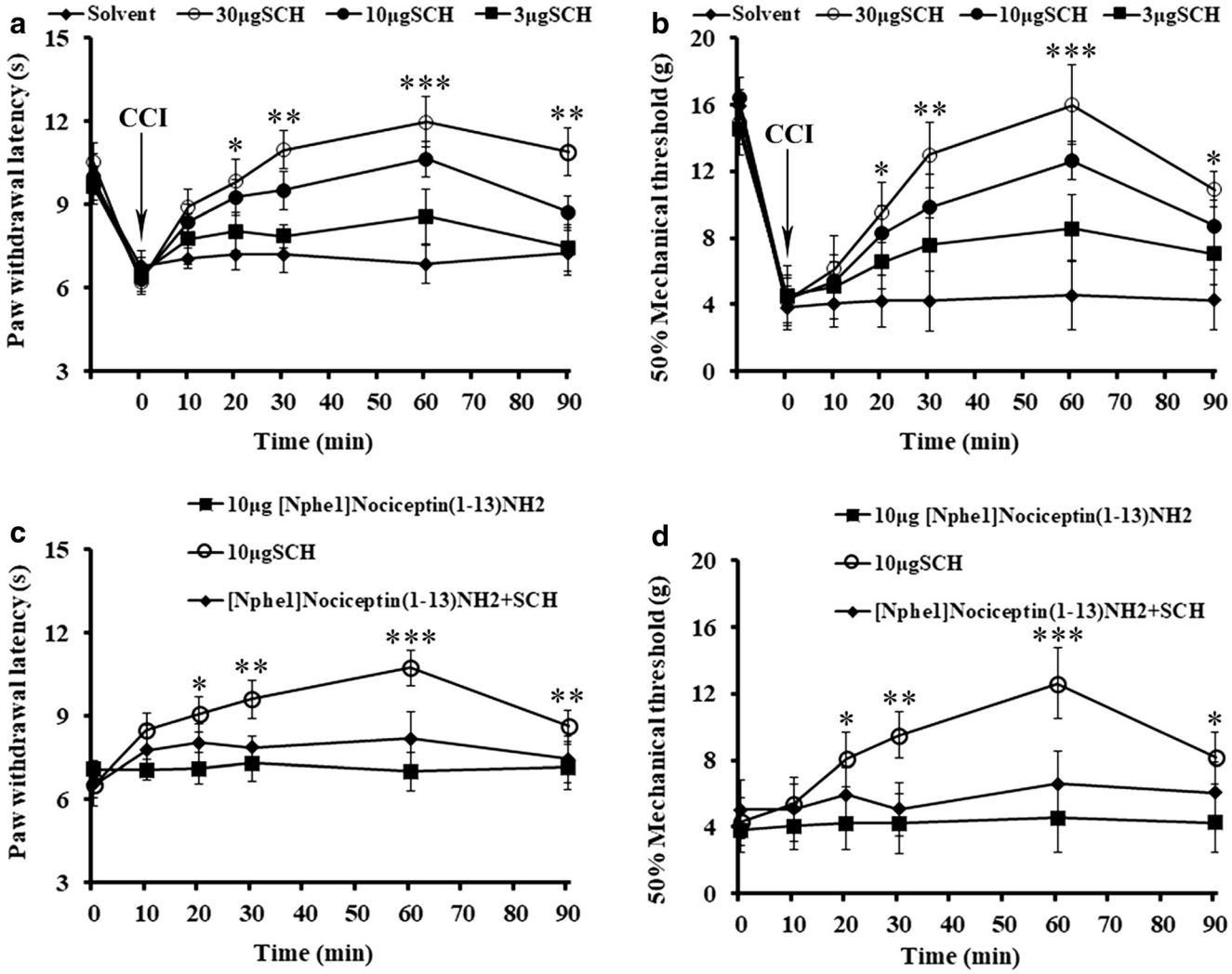

Fig. 1 ORL_1 mediated SCH221510 analgesia in CCI rats. Intrathecal SCH221510 (3, 10, and 30 μg) analgesic effects on PWL to heat (**a**) and 50% mechanical threshold (**b**) on day 7 after chronic constrictive injury to rat sciatic nerve. Intrathecal 10 μg [$Nphe^1$]nociceptin(1-13)NH_2 blocked SCH221510 analgesic effect on PWL to heat (**c**) and 50% mechanical threshold (**d**) on day 7 after chronic constrictive injury to rat sciatic nerve. SCH SCH221510. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. before intrathecal injection

Discussion

The current study showed that intrathecal ORL_1 agonist SCH221510 dose-dependently decreased thermal and mechanical hypersensitivity in hind paw induced by chronic constrictive injury to rat sciatic nerve. The analgesic effect was blocked by ORL_1 antagonist [$Nphe^1$]nociceptin(1-13)NH_2. Intrathecal ORL_1 siRNA decreased the spinal cord ORL_1 mRNA and protein. ORL_1 siRNA did not change CCI-induced thermal and mechanical hypersensitivity. SCH221510 analgesia was blocked with ORL_1 siRNA pre-treatment. The results indicated that spinal ORL_1 activation mediates SCH221510 analgesia on CCI-induced thermal and mechanical hypersensitivity in rats.

Neuropathic pain is usually caused by a lesion or disease of the somatosensory nervous system which impairs quality of life and is often poorly managed. Neuropathic pain animal models are usually caused by peripheral nerve injury through compression or transection to trigeminal nerve or sciatic nerve (Bennett & Xie, 1988; Decosterd & Woolf, 2000; Xu, Aita, & Chavkin, 2008). Chronic constrictive injury to sciatic nerve is one of the most used animal models to study neuropathic pain. Meanwhile, a variety of drug treatments have been recommended including antidepressants, Tramadol, and opioids but more effective treatments are still needed. Morphine was less effective in treating neuropathic pain shown in both clinics and animal studies. μ-Opioid receptor was downregulated and opioid receptor binding was reduced in the spinal cord after peripheral nerve-injury-induced neuropathic pain (deGroot, Coggeshall, & Carlton, 1997; Zajac, Lombard, Peschanski, Besson, & Roques, 1989). However, as a member of opioid receptor family, ORL_1 mRNA and protein were found in the central nervous system (Houtani, et al., 2000; Pettersson, Sundler, & Danielsen, 2002), and the animal

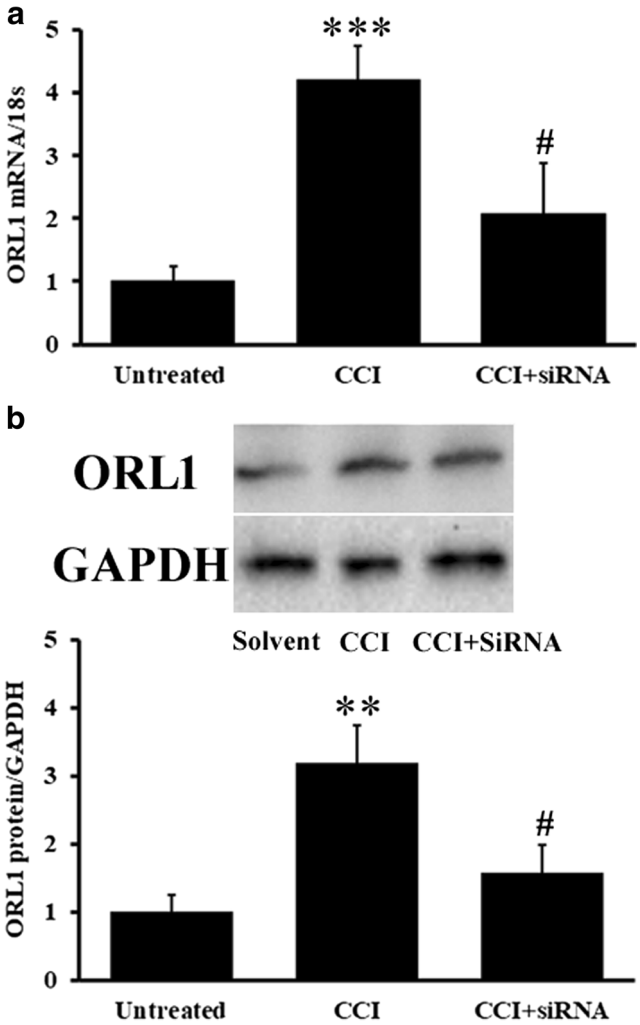

Fig. 2 ORL_1 siRNA decreased spinal mRNA and protein increase by CCI. **a** Histogram showed that CCI increased ORL_1 mRNA compared to untreated. Intrathecal ORL_1 siRNA blocked mRNA increase. **b** Histogram showed that CCI increased ORL_1 protein compared to untreated. Intrathecal ORL_1 siRNA blocked protein increase. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. untreated group; $^{\#}p < 0.05$ vs. CCI group

models of neuropathic pain increased ORL_1 in brain and dorsal root ganglion (Chen & Sommer, 2006; Ma, Xie, Dong, Wang, & Wu, 2005). Spinal ORL_1 mRNA increase shown by RT-PCR indicated that ORL_1 was upregulated after CCI in rats (Briscini, Corradini, Ongini, & Bertorelli, 2002). Our results showed that both ORL_1 mRNA and protein were increased in the spinal cord after CCI injury, suggesting that spinal ORL_1 was modulated differently than μ-opioid receptor in neuropathic pain. However, thermal and mechanical hypersensitivity were not blocked when spinal ORL_1 was not reduced in CCI rats. Our result showed that intrathecal injection of ORL_1 siRNA significantly lowered CCI-induced ORL_1 mRNA and protein increase in the spinal cord 7 days after injection. CCI-induced thermal and mechanical hypersensitivity were also not blocked by intrathecal ORL_1 siRNA. Our result was agreed by another report that intrathecal injection of ORL_1 antagonists alone did not affect CCI-induced thermal and mechanical hypersensitivity (Obara, et al., 2005). In addition, ORL_1 deletion did not block CCI-induced thermal and mechanical hypersensitivity in mice (Bertorelli, et al., 2002; Nishi, et al., 1997). Along with the previous reports that ORL_1 receptor agonists displayed antinociceptive effect in CCI-induced neuropathic pain (Courteix, et al., 2004; Hayashi, et al., 2010), the current study suggested that spinal ORL_1 activation contributed to SCH221510 analgesia on thermal and mechanical hypersensitivity instead of ORL_1 upregulation probably due to lack of activation by endogenous agonist. The mechanisms remain to be determined.

Numerous studies showed that ORL_1 participated in pain process of different pain models in both rodents and primates (Chen & Sommer, 2007; Courteix, et al., 2004; Ko & Naughton, 2009). ORL_1 activation inhibited windup and reduced glutamatergic transmission induced by sciatic nerve injury in the spinal cord, suggesting that ORL_1 could be a novel molecule targeting neuropathic pain (Faber, Chambers, Evans, & Henderson, 1996; Stanfa, Chapman, Kerr, & Dickenson, 1996). ORL_1 upregulation itself did not

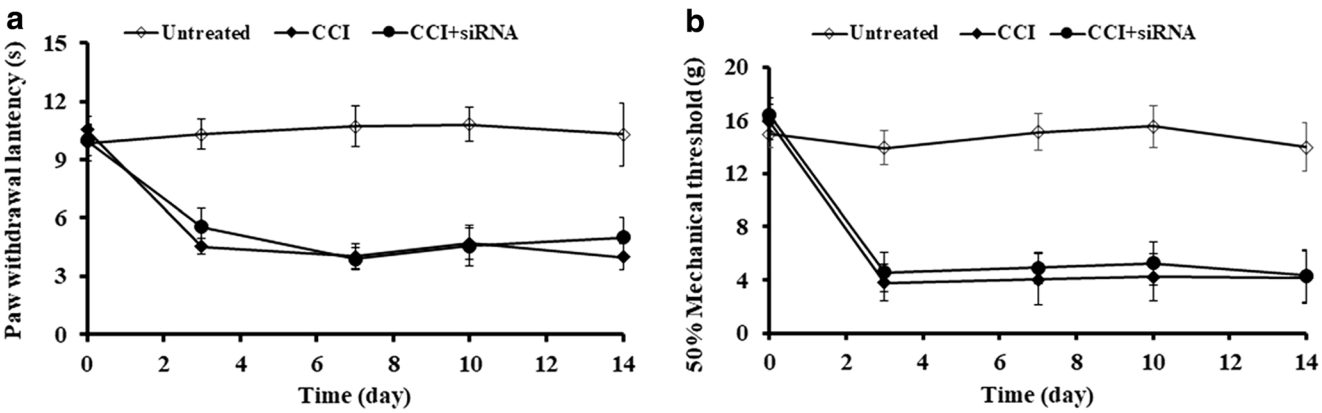

Fig. 3 Effect of ORL_1 siRNA on neuropathic pain. Intrathecal ORL_1 siRNA did not block thermal (A) and mechanical (B) hypersensitivity induced by CCI

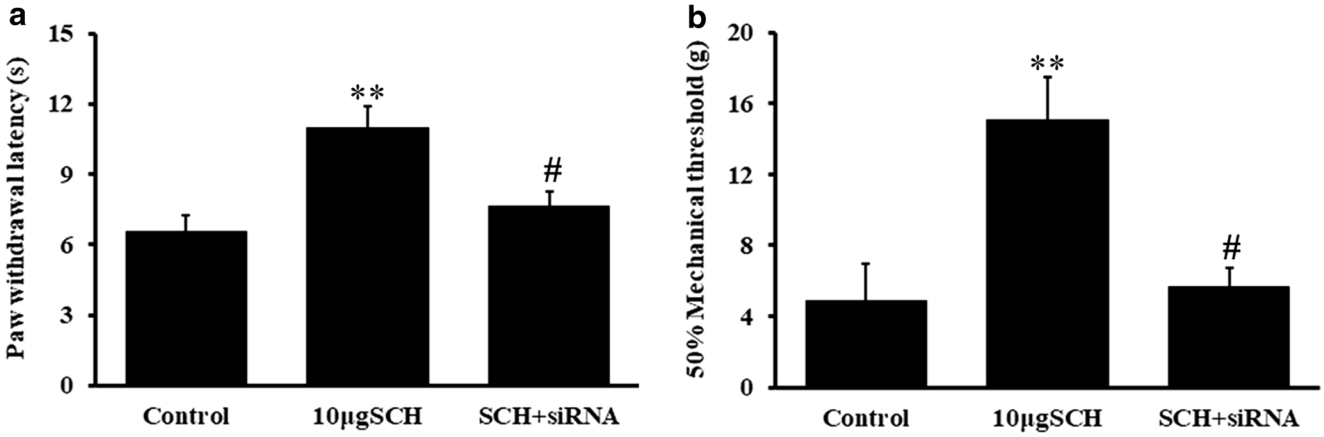

Fig. 4 ORL_1 siRNA blocked SCH221510 analgesia in CCI rats. All three groups had CCI surgery. Intrathecal 10 μg SCH221510 significantly increased PWL (**a**) and 50% mechanical threshold (**b**) when compared to control (CCI only) group. Intrathecal ORL_1 siRNA blocked SCH221510 analgesia on CCI-induced thermal (**a**) and mechanical (**b**) hypersensitivity. SCH SCH221510. $^{**}p < 0.01$ vs. control (CCI only) group; $^{\#}p < 0.05$ vs. CCI plus SCH221510 group

change CCI-induced hypersensitivity but provided increasing number of acting sites for ORL_1 agonists to perform the analgesic effect in neuropathic pain (Obara, et al., 2005; Rizzi, et al., 2015; Sukhtankar, et al., 2013). Our results showed ORL_1 mRNA and protein increase in the spinal cord but did not locate which compartments the increases were. ORL_1 agonists bound to membrane ORL_1 inhibited nociceptive neuron via extracellular signal-regulated kinase (ERK)-dependent non-genomic mechanisms and gated rectifying potassium channels (Ikeda, et al., 1997; Small, Nag, & Mokha, 2013). The mechanism of spinal ORL_1 activation analgesia in neuropathic pain is to be determined.

Mounting ORL_1 receptor agonists including peptide and non-peptide agonists have shown that the antinociceptive action at spinal level was through pharmacological activation of ORL_1 (Hayashi, et al., 2010; Obara, et al., 2005). Hayashi et al. reported that a compound HPCOM, one of the ORL_1 agonists, showed robust antinociceptive effect at spinal level with enhanced bio-pharmacological availability, suggesting ORL_1 agonist as a potential systemically potent new-classic analgesic (Hayashi, et al., 2010). Another non-peptide ORL_1 agonist, SCH221510, potentiated analgesic effect of μ-opioid receptor agonist in primate through systemic administration (Cremeans, et al., 2012). SCH221510 was orally active and showed its antinociceptive effect in visceral pain during treating gastrointestinal diseases, suggesting an easier administration drug for pain treatments (Sobczak, et al., 2014). Intrathecal injection of SCH221510 reduced thermal and mechanical hypersensitivity induced by nerve injury in mice and it also displayed the antinociceptive effect in primate (Sukhtankar, et al., 2013). Our results showed that intrathecal SCH221510 dose-dependently reduced CCI-induced thermal and mechanical hypersensitivity in rats which was blocked by intrathecal ORL_1 antagonist or siRNA, suggesting that ORL_1 activation mediates SCH221510 analgesia in neuropathic pain.

In conclusion, the present work characterizes a new ORL_1 receptor agonist, SCH221510, in neuropathic pain. The study proves ORL_1 expression increased at both mRNA and protein levels and utilizes siRNA technique to suppress ORL_1 translation beside receptor antagonists.

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Availability of Data and Materials The authors will provide data upon request.

Author Contributions QW designed the experiment, carried out the study, and drafted the manuscript. LL carried out the experiments.

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Compliance with Ethical Standards

Ethics Approval The experiment was approved by the Animals Care and Use Committee of Luoyang Medical College.

Consent for Publication The authors have consent to publish the study.

Competing Interest The authors declare that they have no competing interests.

Abbreviations ORL_1, opioid receptor like 1; CCI, chronic constrictive injury; N/OFQ, nociceptin/orphanin FQ; PWL, paw withdrawal latency; PB, phosphate buffer

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